

ABSTRACTS OF PRESENTATIONS

(Alphabetical order by Presenters)

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Al-Attar, Salam. Department of Biology, University of Western Ontario.

MRI technique quantitatively unravels mysteries underlying monogenic forms of diabetes type 2

Single gene diseases are good model systems to dissect the pathways underlying the complex polygenic forms of common disorders such as cardiovascular disease and diabetes. One such human monogenic model system is familial partial lipodystrophy of the Dunnigan-type (FPLD), which occurs in two molecular forms: FPLD2 and FPLD3, due to mutations in LMNA and PPARG, respectively. These single base pair mutations result in adverse effects, beginning with the loss of subcutaneous fat in the extremities and leading to the development of insulin resistance and diabetes with age.

Genome sequencing technology has uncovered the molecular basis of these monogenic disorders, but their phenomic assessment remains relatively crude. Through the use of magnetic resonance imaging of FPLD patients, this study developed a method of quantifying the fundamental FPLD anthropometric phenotype; particularly adipose tissue distribution in the mid-calf, mid-thigh and abdominal regions. MRI images reveal a difference in the amount of subcutaneous adipose tissue in these regions of FPLD patients in comparison to those of normal controls. Furthermore, FPLD2 patients appear to have a significantly reduced amount of subcutaneous adipose tissue compared to FPLD3 patients, in contrary to previous correlations made with the metabolic consequences associated with each FPLD type. The semi-automated adipose imaging of the lower extremity and abdomen regions in this study can provide informative differences between individuals of various genotypes with respect to lipodystrophy and represents a potentially useful tool for extended quantitative phenotypic analysis of other genetic metabolic disorders.

Alkema, Michael and Chow-Fraser, P. McMaster University, Dept. of Biology.

Examining the use of a small forest fragment by avian guilds in Southern Ontario

The decline in avian populations in North American is not well understood. Reduction of habitat is believed to be the main cause of the decline. Only small islands of forests remain in the sea of human development. The bird population of a small forest of Ruthven National Historic Park was monitored over a ten year period (1995-2005). Mist nets were used to sample the bird population according to the Canadian Migration Monitoring Network protocols. Avian species distribution and abundances were examined to understand the usefulness of a small forest fragment. The health of the avian community was measured in two ways. First, the proportion of birds that returns to the forest is one indication. A second indication is the reproductive status and proportion of fledging birds of the local population. Bird species were grouped into guilds based on their preference of habitat, breeding habitat, nesting location and migratory strategy. Due to the small size of the forest, edge specialist dominated the avian population. Our results indicate a homogenizing of avian species to edge specialists and an overall decline in population in the short term. Understanding the use of forest fragments by bird species will help conservation efforts for the declining avian populations.

Al-Rifai, Tahani, Department of Biology, McMaster University.

The comparison of CERVUS Vs. PARENTE Computer programs, using Microsatellite Markers from Smooth-Billed Ani

The Species, Crotophaga Ani is a joint nesting, plural-breeding bird. The smooth billed ani is a member of this subfamily and usually forms breeding groups of two to seventeen individuals sharing single nest containing as many as thirty six or more eggs. Anis exhibit reproductive skew. Ani's reproduction system varies from equable, where reproduction is shared more equally among same sex group to monocratic where a single individual occupies all the reproduction in a certain group. Anis also show various unique behaviors such as egg burial and egg tossing. The presence of such behavior and reproduction skew make it rather difficult to interpret parentage analysis. However, the bioinformatics revolution in biology has allowed scientists to collect a huge amount of information about what seemed to be impossible to do thirty years ago. This study aims to use two computer programs (CERVUS and PARENTE) to assist in the parentage analysis of smooth billed ani and compare their accuracy in doing so. Samples of DNA for adults and offspring were collected in the field, extracted and amplified using four microsatellite primer sets. Parentage and relatedness among individuals were assigned using two computer programs (CERVUS and PARENTE) which revealed great variety in their results.

Andersen, S. Faculty of Forestry and the Forest Environment, Lakehead University.

Fungal Associates of the Bronze Birch Borer on Urban Birch.

Birch decline is a serious problem commonly encountered in urban environments. It is often initiated by stress such as drought or poor growing conditions and commonly results in infestations by the bronze birch borer (*Agrilus anxius*). These wood-boring insects lay their eggs in crevasses on the bark with the resulting larvae producing galleries which girdle the tree. Death is quick, occurring over a period of one or two years if the tree cannot defend itself from the attack. In all cases it has been assumed that the death is due to the activity of the beetle. Although several studies have been published on fungal associates of wood-boring beetles, none have been published to date on fungal associates of the bronze birch borer. Isolations were taken from bronze birch borer-infested white birch (*Betula papyrifera*) in order to determine whether any fungal associations were present. A total of eight trees were removed from the urban centre of Thunder Bay during the summer of 2005 and used in this study. Areas on the trees that were visibly infested with the bronze birch borer were cut into small logs and bagged. Isolations were taken from both the beetle galleries and beetle frass directly from the logs and in addition, larval washings were performed on any larva that had been removed. Many fungi grew out in culture, including species in the genera *Melanconium* and *Cytospora* which are known to be associated with cankers and/or decline in birch. This could suggest that fungi found on birch that were once thought to be secondary pathogens may actually play a more serious role in birch decline. At this time no guarantee can be made that these fungi are specifically associated with the bronze birch borer as further studies are necessary in order to investigate this potential relationship.

Anstey, Michelle I., MacLean, Allyson and Finan, Turlough M. Department of Biology, McMaster University.

Characterization of the Regulation of Protocatechuate genes in *Sinorhizobium meliloti* by LysR-type transcriptional Regulator, PcaQ

Sinorhizobium meliloti is a gram-negative alpha-proteobacteria that forms a symbiotic relationship with the plant *Medicago sativa*, in order to fix atmospheric nitrogen (N_2) to ammonia (NH_4^+) that the plant can utilize as a nitrogen source. Protocatechuate (PCA) is an aromatic compound produced by plants that can be utilized by *S. meliloti* as a carbon and energy source through the catabolic pathway encoded in the *pcaDCHGB* and *pcaIJF* operons. Analysis of the LysR-type transcriptional activator of the *pcaDCHGB* operon, PcaQ, was performed using electrophoretic mobility shift assays. PcaQ was found to bind the *pcaDCHGB* operon with many protein:DNA shifts forming as increasing amounts of PcaQ are added to the binding reaction. PcaQ was also found to bind to the promoter region of a putative protocatechuate specific ABC transporter, also showing the ability to form multiple PcaQ:DNA shift complexes as additional PcaQ is added to the reaction. Description of PcaQ as a regulator of an ABC transport system is a novel discovery, and binding of PcaQ to this region further confirms this discovery. Further it was shown through FPLC size exclusion chromatography that PcaQ elutes at a size of 149kDa, suggesting it forms a tetramer, a common property of LysR-type regulators.

Alwaysheh, Abudi. Department of Biology, University of Western Ontario.

Glutathione Depletion and S-Glutathionylation of the Metabolic Proteins GAPDH and ICDH in Murine Hepatoma Hepa 1c1c7 Cells After Exposure to the Oxidant Stressor t-Butyl Hydroperoxide

Cell damage induced by oxidative stress and reactive oxygen species (ROS) has been implicated in several human diseases including aging, alcohol-mediated organ damage, neurodegenerative diseases, many types of cancers, cardiovascular diseases, and UV-mediated skin disorders. Native (endobiotic) or foreign (xenobiotic) toxic chemicals, can be lethal as a result of direct or indirect generation of intracellular ROS. T-Butyl Hydroperoxide (TBHP) a xenobiotic, oxidizes proteins with reactive cysteine thiol residues (PSH); (ionized at physiological pH) to sulphenic acids (PSOH) which react non-enzymatically with glutathione (GSH), forming reversible protein glutathione mixed disulfides (PSSG), a process termed S-glutathionylation. In the absence of GSH, the sulfinic acid is further oxidized by ROS to inactive protein sulfinic (RSO₂H) and sulfonic (RSO₃H) densities that are inactive. We plan to study S-glutathionylation of two mitochondrial metabolic proteins, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and isocitrate dehydrogenase (ICDH), in subcellular fractions of murine hepatoma Hepa 1c1c7 cells after exposure to, TBHP. It has recently been reported that S-glutathionylation of these two proteins at single specific reactive cysteine thiol residues, results in attenuation of enzyme activity.

Barclay, M.E.* and MacDougall-Shackleton, E.A. Department of Biology, University of Western Ontario.

Maternal condition and adaptive sex ratio manipulation in Song Sparrows (*Melospiza melodia*)

The Trivers-Willard hypothesis suggests that in certain situations, the ability of a female to manipulate the sex of her progeny according to her condition would be favoured because it would increase her reproductive success. For example, if a female is in good condition, she should produce more of the sex whose success in reproduction is most dependent on condition and therefore more variable. Song sparrows (*Melospiza melodia*) are a species that meet at least some of the conditions needed for adaptive sex allocation to be advantageous to them. Males require more parental investment in order to survive than females, but if males are in good condition they have the opportunity to mate with several females because they occasionally have extra-pair copulations. We hypothesized that female song sparrows in poor condition would have female-biased sex ratios and females in good condition would have male-biased sex ratios. In order to test this hypothesis, the body condition of 22 mothers from a song sparrow population was determined as well as the sex of their offspring. The heterophil/lymphocyte ratio, body fat, tarsus length asymmetry, and mass to right tarsus length residual was determined for each mother and used in a principal components analysis to calculate the body condition of each mother. The sexes of 98 nestlings from 29 broods, were determined by the amplification of the chromobox-helicase-DNA-binding (CHD) sex chromosome gene by PCR. We found that the females in this population of song sparrows appear to manipulate their offspring sex ratios according to their condition. As predicted, the better the condition a female was in, the more male-biased her offspring were.

Barker, Nicole K., and Scott M. Ramsay. Department of Biology, Wilfrid Laurier University.

Can white-throated sparrows be used as an indicator species?

Research on species at risk is important for conservation, but because populations of these species are necessarily low, studying them directly is often quite difficult. It is therefore useful to find common and easily-studied species whose population dynamics can be used as indicators of rare or threatened species. We are looking at data from various sources to assess if the white-throated sparrow (WTSP) can be used as one such species; they are a common edge species and their population fluctuations likely indicate changes in forest structure and land use. A comparison between data collected in 2005 with previously collected data indicates that WTSP populations have shifted in various areas of Algonquin Park since the 1980's. The annual Breeding Bird Survey (BBS), which provides census data for bird species in North America, indicates population changes since 1966; preliminary analysis of these data reveals varying associations between populations of WTSPs and other birds. We will also be examining aerial photographs to quantify the relationship between WTSP abundance and vegetation characteristics. Developing a complete understanding of the relationships between WTSPs, other species, and vegetation characteristics will allow for predictions to be made based upon simply studying WTSPs. WTSPs are easy to hear, identify, and study, so their use as indicators of other species or habitat quality will save time, money, and energy while studying species at risk. Findings from this study may new uses for citizen science (BBS) and highlight the importance of studying common and easily-detected species. Based on these findings, census data for the WTSP can be used as a starting point for targeting areas of concern for conservation and management.

Bogart, Sarah J. and Cholewa, Ewa. Department of Biology, Nipissing University.

Do sclereids facilitate the survival of *Eriophorum vaginatum* L. growing in metal contaminated areas?

Eriophorum vaginatum, a tussock forming sedge, thrives in cold, nutrient poor environments and metal contaminated industrial areas. The survival of *E. vaginatum* might be correlated with the presence of lignified sclerenchyma cells, or sclereids. Sclereids present in *E. vaginatum*'s corm are alive at maturity and associated with vascular bundles. Previous study implied that these sclereids may be involved in internal nutrient recycling. It has also been demonstrated that *E. vaginatum* accumulates and stores metals (e.g. ¹³⁷Cs, Pb, Cu, and As). We are determining whether or not sclereids are metal detoxification centers within the corm of *E. vaginatum*. Moreover, sclereids were discovered in the closely related, but non-tussock forming, *E. viridicarinarum* Engelm. (Fern.). *E. viridicarinarum* was also found in nutrient challenging and contaminated sites. Sclereids differ in this species from those of *E. vaginatum* in colour and potentially in composition yet, position, size and morphology are similar. Sclereids in both species are localized on the adaxial side of vascular bundles in the corm which implies a function in addition to mechanical support. We are currently examining the metal composition of sclereids from *E. vaginatum* collected from contaminated sites near Sudbury, ON, employing ICP-MS. This analysis will determine metal content of sclereids and may shed light on the role of sclereids in the mechanism of plant survival in contaminated environments. Results from this study may lead to the use of *E. vaginatum* as a bioremediative plant throughout the northern hemisphere.

Bolton, Frances L., Albrecht I. Schulte-Hostedde, and Jacqueline D. Litzgus. Department of Biology, Laurentian University.

Effect of body condition on the reproductive output of spotted turtles (*Clemmys guttata*) from South Carolina.

The body condition of an animal reflects energetic state such that an animal in good condition will have more energy reserves than one that is in poor condition. Body condition can be estimated as the positive residuals from a correlation between body length and mass. Since body condition reflects an animal's energetic state, it should be related to reproductive output since energetic reserves limit the amount of energy that an animal can put into reproduction. Using spotted turtles (*Clemmys guttata*) as a model system and three years of field data from a South Carolina population, we tested the hypothesis that animals in good condition will have higher fitness than those in poor condition. The effect of body condition on the reproductive output of female spotted turtles was examined using three types of analyses and several reproductive variables, including clutch frequency, clutch size and egg size we predicted that (i) females in good condition will have a higher clutch frequency than those in poor condition; (ii) females in good condition will have larger clutch sizes than those in poor condition; and (iii) females in good condition will have larger eggs than those in poor condition. In the first analysis, each clutch of eggs was treated as an independent unit, and we found a negative relationship between body size and all of the reproductive variables studied. The second analysis treated each year as an individual unit, and we found a positive relationship between body condition and some measures of egg size, and a negative relationship between body condition and clutch frequency and average clutch mass. The third analysis treated each female over the entire three-year study as independent units, and we found a positive relationship between body condition and all reproductive variables studied. The results of this study did not fully support our hypothesis as females in good condition did not always produce more eggs and/or more clutches than those in poor body condition.

Bourassa, Jessica. Department of Biology, University of Western Ontario.

Characterization of a morphological cline in two subspecies of *Limenitis arthemis*, *Limenitis arthemis arthemis* and *Limenitis arthemis astyanax* (Nymphalidae: Limenitidinae) from Southern Ontario

Hybrids between the white admiral (*Limenitis arthemis arthemis*), and red-spotted purple (*Limenitis arthemis astyanax*) butterflies occur in a zone through southwestern Ontario south of the Canadian Shield, and occasionally also in southeastern Ontario. The white admiral is most common in northern Ontario whereas the red-spotted purple is common only in extreme southwestern Ontario. A cline exists along a north-south gradient across their geographic range in Ontario. I used a historical (1970's and 1980's) collection of butterflies from several locations along the cline. Individuals were photographed and I measured various wing characteristics to determine the shape and location of the cline. The results demonstrated that several traits varied along the cline. My study provides valuable information on the location and shape of the cline, information that is valuable for future investigation of why the cline exists in the first place and how it is maintained with regard to natural selection and gene flow.

Brassard A.*, and Mallory F.F., Department of Biology, Laurentian University.

A Comparison of The Gastro-intestinal Tract And Body Mass Ratio In Mammals Of North America

The primary objective of this research was to compare the ratio of the digestive tract and the animal body mass in North American mammals. A total of 83 specimens were necropsied and compared at the Species, Family and Order taxonomic level. The mean average of gastro-intestinal (GI) tract represents 4.56 % of the body mass in mammals. Sample sizes at the species level were too low to analyze statistically. Variation was observed within Families and Orders. At the Family level, Ovidae had the lowest ratio and Bovidae the largest for the order of Artiodactyls (foregut fermentors). Sciuridae had the lowest ratio and Erethizonidae the highest ratio in Rodents and Lagomorphs (hindgut fermentors). For Insectivores and allies, Soricidae had the lowest ratio while Vespertilionidae had the highest ratio. In Carnivores, Canidae had the lowest ratio and Ursidae the highest. Regression analysis indicated that Herbivores (Artiodactyls with Rodents and Lagomorphs) had the same ratio (0.0524) which was also the highest. Insectivore and allies (0.0444) and finally Carnivores (0.0344) followed. These results are discussed in relation to foraging strategies and food palatability.

Buckner, A. and Lafrenie, R. Laurentian University.

The effects of Raf clones on cell death in 293T cells

The Raf kinases work at the entry point of the mitogen-activated protein kinase pathway which controls cell division and cell death. Raf-1 is known to be an oncogene and is hyperactive in 30% of all transgenic tumours. A mutant activated form of Raf-1 (Raf-CA) is known to promote cell growth, while the mutant inactive form (Raf-DN) may promote cell death. To test this, we examined the effects on cell death following transfection of human embryonic kidney 293T cells with Raf-CA, Raf-DN, full length Raf-1, and control plasmids. Samples were collected on days 1, 3, 5 and 7 after transfection and were subjected to survival analysis (trypan blue staining), immunoblot analysis for markers of cell growth or apoptosis, DNA fragmentation studies, and flow cytometry analysis for apoptosis. Survival of the cells following transfection showed differences between the transgenes (two-way analysis of variance showed main effects for the clones and days, and showed an interaction of clones by days). Cells transfected with Raf-CA, the constitutively active form, showed increased cell growth while cells transfected with the inhibitory form, Raf-DN, showed decreased cell growth and increased cell death. The full length Raf-1 clone was not significantly different from the control GFP. The goal of these studies is to understand the components driving the uncontrolled growth and decreased death of cancerous cells that could give us insight on how to more effectively treat cancer patients.

Buttigieg, Holly², Michael Kawaja³, and Margaret Fahnstock^{1,2}. ¹Department of Psychiatry & Behavioural Neurosciences and ²Department of Biology, McMaster University, Hamilton, ON, L8N 3Z5, Canada; ³Department of Anatomy & Cell Biology, Queen's University, Kingston, ON, K7L 3N6, Canada.

In Search of a Function; The Biological Activity Of ProNGF *In Vivo*

There is disagreement in the literature over whether pro-nerve growth factor (proNGF) is neurotrophic or apoptotic (Fahnstock et al., 2004). In this study, proNGF levels were examined in cerebellar and hippocampal tissue of genetically modified mice. Tissue samples were obtained from transgenic mice over-expressing the NGF gene under the control of the glial fibrillary acidic protein (GFAP) promoter (cerebellum n=8, hippocampus=5), and wild type controls (cerebellum n=9, hippocampus=5). These NGF over-expressing mice exhibited a robust ingrowth of sympathetic fibers and no cell loss of the sympathetic ganglion neurons (Kawaja & Crutcher, 1997). Because of the cell survival and the controversies found in the literature regarding proNGF, it was important to determine whether the expressed product was NGF or proNGF. A blinded western blot analysis was carried out to compare the NGF levels between transgenic mouse samples and their wild type controls. We found that mature NGF was expressed at very low levels, whereas proNGF appeared at ample levels for quantification. Further analysis demonstrated a four fold increase in proNGF protein in mice over-expressing the NGF gene in comparison to controls. The over-expression of NGF in cerebellar and hippocampal tissue results in an increase in proNGF rather than mature NGF levels. Together with the robust axonal growth and lack of neuronal death in the sympathetic ganglion in these animals, our results are consistent with proNGF exhibiting neurotrophic activity *in vivo*.

Castonguay, Erika. Department of Biology, University of Western Ontario.

Nest mortality of deer mice in Southwestern Alberta

Small mammals have the potential for large litters and high reproductive rates; however populations rarely grow at the rate dictated by their reproductive potential. Nest mortality causes the number of recruits in relation to the number of offspring born to vary greatly in populations. Many studies have observed the effects of nest mortality on populations and found that it influences population growth. My study will cover an unexplored aspect of nest mortality to determine which type of nest failure influences population growth more significantly. A nest may be unsuccessful due to the death of the mother resulting in the loss of the litter or due to direct death of the litter as a result of predation, weather or infanticide. I hypothesized that the fate of the mothers will influence recruitment more so than other causes of litter death. The dynamics of nest mortality was examined based on trapping records of deer mice (*Peromyscus maniculatus*) in Kananaskis, Alberta at Grizzly Creek and Fortress Mountain from 1991 to 2005. It was found that the populations did not differ significantly per site or per year in any variables of survival, number of recruits, population growth or number of nest mortalities. However, the number of nest failures due to dam's death did differ significantly per year. Other variables were taken into account and observed that the increased number of dam's death was a result of years of flooding. The main findings of my results were that both the failure of nests due to mother's death and the failure of nest due to other causes influenced population equally. Not one variable was more influential on the number of nest mortalities and on the population dynamics.

Chahine, Sarah. Department of Biology, McMaster University.

The Use of Egg Characteristics and DNA Analysis in Determining Maternity in Communal Clutches of the Smooth-billed Ani

Smooth-billed ani exhibit a rare polygynandrous reproductive system with groups containing several male and female breeders. Female reproductive strategies involve, among others, leaving the group, varying clutch size, egg ejection and infanticide. Egg characteristics have been used to determine egg maternity for birds in situations where two or more females lay eggs in a single nest. We assessed the applicability of egg characteristics ascribing egg ownership in communal clutches of Smooth-billed ani. We used both egg characteristics and DNA analysis to determine maternity in communal clutches of the Smooth-billed. Also, based on their egg characteristics, we conducted discriminate function analyses to classify eggs to their correct mothers. The correct maternity was determined by DNA analysis using egg swab. We used Image J program for egg characteristics analysis, where we looked at the volume and the shape of the egg using the image of the egg. We used blood samples from egg swabbing and analyzed them using Polymerase chain reaction (PCR) and acrylamide gel electrophoresis to identify individual females' eggs in joint nests.

Charbonneau A., Beckett P.J., and Courtin G.M. Department of Biology, Laurentian University.

Proposed Restoration of a Northern Ontario Diamond Mine: Drought Tolerance of Cottongrass (*Eriophorum vaginatum*)

A diamond mine 90km west of Attawapiskat in northern Ontario will produce waste of fine particulate kimberlite (FPK) and extracted peat. To initiate ecosystem reconstruction and to encourage native peatland species to re-establish and thrive, cottongrass (*Eriophorum vaginatum*) has been proposed as a nurse species. Since the proposed restoration sites are likely to be better drained than the existing wetland, the drought tolerance of cottongrass was explored. This experiment investigated four different substrates in order to determine the best substrate suitable for the growth of cottongrass; 100%peat, 50:50 peat:FPK, 75:25 peat:FPK, and 100%FPK, and subjected to natural drying conditions over a three month period. Cottongrass with cylindrical leaves does not wilt visually and so, to detect when *E. vaginatum* has exhausted the available water within the substrate, the weight loss over time was recorded. All columns were sealed so that the only method the water could get out of the soil was through transpiration from the plant. In order to determine the difference in drought response between the four substrates, root and shoot mass, soil water content, and microclimate were considered. One way Analysis of Variances showed that there were no differences in the shoot mass for each substrate; however, there was a difference in root mass for each substrate. A difference in soil water content for each substrate may suggest that even though water loss from shoots is comparable, the roots react differently to each substrate, and may be affecting growth performance. Thus, it appears that cottongrass is reasonably drought tolerant and makes it a good potential companion species for peatland restoration.

Cheng, Amy W.H., Zhu, Xu-Dong and Wu, Yili. Department of Biology, McMaster University.

Does the XPF Endonuclease have a Role in Telomere Maintenance of Human ALT Cells?

Telomeres are structures located at the ends of linear chromosomes. They are arrays of repetitive TTAGGG DNA sequences accompanied by telomere-associating proteins. Together, they serve to resolve two problems that originate from the end-replication problem of eukaryotic DNA replication: the shortening of DNA length per replication, and the activation of degradative or DNA repair activities upon recognition of opened chromosome ends. The lengths of telomeres are maintained by two known types of Telomere Maintenance Mechanisms (TMM): telomerase-dependent, and telomerase-independent (also known as Alternative Lengthening of Telomeres, ALT). TMM is used by germ cells and cancerous cells and is crucial for the immortalization in human tumorigenesis. ALT is used by ~10% cancerous cells as the telomerase-dependent mechanism is predominantly found in germ cells and ~90% of cancer cells. Although the mechanisms employed by ALT cells are still poorly understood, there is ample evidence indicating that homologous recombination is involved in the telomere maintenance in ALT cells. This research project is aimed to reveal whether the endonuclease XPF takes part in the telomere maintenance in ALT cells. Previous studies have shown that the ERCC1-XPF endonuclease complex can be recruited to the telomeres and is required for homologous recombination-mediated double-strand break (DSB) repair, suggesting a potential role for XPF in the homologous recombination of telomere maintenance in ALT cells.

Choi, Alvin S., Schellhorn, Herb E., and Hughes, Andrew H. Department of Biology, McMaster University.

Characterization of the *ymdF* promoter in *Escherichia coli*

Sigma factors are essential for specific recognition of promoter sequences to ensure proper transcription. RpoS is a sigma factor that is highly expressed during the stationary growth phase of *Escherichia coli* and promotes the expression of genes necessary to survive in an environment not favorable for growth. Previously, microarray analysis found that *ymdF* expression was greater in strains expressing RpoS. The purpose of this study is to determine whether *ymdF* transcription is dependent on RpoS by studying the *ymdF* promoter. Through use of *rpoS*⁺ and *rpoS*⁻ strains with pOT1 reporter plasmids, various constructs of the *ymdF* promoter were incorporated into the pOT1 reporter plasmid upstream of a promoterless *gfpUV* gene. Fluorescence from GfpUV expression was measured hourly from cells grown in Luria Bertani media. It was found that the *rpoS*⁺ strain possessing the full length promoter within the pOT1 plasmid displayed growth phase dependent expression of GfpUV. Fluorescence readings were taken from cells grown over a period of 3 days and it was found that the *rpoS*⁺ strain containing the reporter with the full promoter construct displayed expression 3-5 fold higher than the *rpoS*⁻ strain containing the same construct. However, when pOT1 constructs containing only RpoS binding sites were tested, fluorescence was found to be below pOT1 control strains. Reverse transcription PCR (RT-PCR) was performed in both *rpoS*⁺ and *rpoS*⁻ strains in order to quantify *ymdF* mRNA. It was found that *ymdF* transcript was present in greater amounts in the *rpoS*⁺ bacteria supporting the results found from the reporter assays with the strains containing the full promoter. This study supports the findings from previous microarray analysis that RpoS increases *ymdF* expression; but cannot conclude that RpoS is dependent for *ymdF* transcription.

Crocker, Brent and Lesbarrères, David. Biology Department, Laurentian University.

Migration or history: explanations into changes in genetic diversity of *Rana temporaria* populations in Southern Sweden

Many species exhibit a spatial structure, where local populations are subjected to various levels of gene flow depending on habitat connectivity. This type of spatial structure is evident in amphibian populations due to the heterogeneity within the landscape, as most amphibians are closely tied to water. These local populations undergo differential dynamics including mortality and migration rates. Through time, local extinctions as well as periods of recolonization are common in these taxa, but genetic consequences have seldom been investigated. Using genetic approaches I will investigate variation in genetic diversity among *Rana temporaria* populations sampled at two different times in southern Sweden. The aim of this study is to estimate how both history and spatial heterogeneity are related to changes in genetic diversity. Eleven populations, *i.e.* 578 individuals, were sampled in 2000 and 2003 and their genetic variation was estimated using 8 microsatellite loci. All the measures used (mean number of alleles, observed heterozygosity and Nei's unbiased gene diversity) presented a significant variation from 2000 to 2003. Migration patterns among populations and landscape fragmentation are the likely explanations for the genetic diversity variation observed. Population dynamics (*i.e.* local extinction processes) are also playing a role in the levels of variation. Such a study allowed us to better understand the extinction-colonization processes that occur in amphibian populations. This could be used in long-term conservation management in the context of the global amphibian population decline.

Cuthbert, Jessica L. and Mennill, Daniel J. Department of Biological Sciences, University of Windsor.

The Duetting Behaviour of Pacific Coast Plain Wrens (*Thryothorus modestus*)

While the vocalizations of temperate avian species have been widely characterized, those of tropical birds remain largely understudied. Duets are complex vocalizations observed in many tropical birds, but few temperate birds. Duets are antiphonal vocalizations performed by the male and female of a pair and occur in widely varying forms in more than 220 species worldwide, yet their function remains unknown. As do many other *Thryothorus* wrens, Plain Wren (*Thryothorus modestus*) pairs sing highly complex duets. We analyzed the songs of birds from 10 different territories of Pacific coast Plain Wrens inhabiting the mature humid forest of Costa Rica, and characterized the acoustic structure of vocalizations produced by both males and females. We found that males produce two distinct types of songs, termed "I-phrases" and "B-phrases", distinguished mainly by their frequency ranges, with I-phrases typically sung in the 2031 to 9135 Hz range, and B-phrases sung in the 1619 to 7219 Hz range. We also found that females produce one type of song, termed "A-phrases", which they rarely sing as solos, but contribute to duets. Duets were usually begun by a male I-phrase, which was answered by a female A-phrase. Males then switched to their B-phrase, and the ensuing duet followed a cyclic pattern of A-B repeats. The three phrases sung by Plain Wrens showed remarkable similarity to those of their sister taxa, Canebrake Wrens (*Thryothorus modestus zeledoni*), which inhabit the scrub forest of the

Carribean coast. However, some Plain Wren I-phrases extended into a lower frequency range than those of Canebrake Wrens, suggesting that differences in the acoustic properties of their songs may reflect differences in their habitats. By analyzing the fine acoustic structure of Plain Wren duets, we were able to provide a detailed description of these vocalizations, as well as a compelling comparison to other *Thryothorus* duets.

Cvetkovic, Maja, Wei, Anhua and Chow-Fraser, Pat. Dept. of Biology, McMaster University.

An assessment of anthropogenic impacts on coastal wetlands of Fathom Five National Marine Park: Comparison of present ecological conditions with those in the 1990's

Fathom Five National Marine Park (FFNMP) is located at the tip of Northern Bruce Peninsula, and includes 27 km of shoreline and three main islands. Its location at the junction of Lake Huron and Georgian Bay makes the Park ecosystems very unique and varied. Wetland habitat is rare, and existing wetlands are critical for aquatic vegetation and fish habitat. In this project I evaluate the present wetland quality of four island sites that have minimal human impact, and five mainland sites that are associated with known human activities within FFNMP. Sites were sampled in summer of 2005 for water quality, fish, and plants, and these were used to generate indices of wetland quality according to the Water Quality Index (WQI; Chow Fraser 2006), Wetland Fish Index (WFI; Seilheimer and Chow-Fraser 2006) and Wetland Macrophyte Index (WMI; Croft and Chow-Fraser, unpub. data). Significant differences between island and mainland sites were found for both WQI and WFI scores (Wilcoxon signed rank test). Results from a 1993 plant study were used to generate WMI scores and these were compared with 2005 WMI scores. Similarly, fish data from 1997 were used to generate WFI scores and compared with values from 2005. We found no significant differences (Wilcoxon signed rank test) between time periods for either WMI or WFI scores. Although there was no evidence of human impact based on WMI, the significant difference in WQI and WFI scores between island and mainland sites suggest that human activities have had a measurable negative impact on the quality of wetlands on the mainland. We also speculate that reduced water level through the past decade has negatively affected the plant and fish communities of FFNMP, and this has been most evident for the island sites, which are largely free of human disturbance.

Dambrosi, Sarah and Slawson, Robin. Department of Biology, Wilfrid Laurier University.

Preferential grazing patterns of *Tetrahymena* on indigenous and pathogenic aquatic bacteria

Protozoan grazing on bacteria in the environment is typically thought to reduce the survival of potential pathogens. However, certain bacteria are known to survive inside the protozoan vesicles, enclosing the bacteria within an additional membrane. This membrane could potentially provide a protective environment that will prevent the bacteria from being subjected to negative impacts. For example, in water and wastewater treatments, opportunistic pathogens could survive disinfection strategies, and pose a public health threat. The primary goal of this study is to assess the impacts of protozoan grazing on pathogen survival. Two different environmentally isolated bacteria, one indigenous to the area and an opportunistic pathogen, *Aeromonas* spp., as well as a transient pathogenic organism, *Salmonella* spp., will be used as model organisms to test survival abilities of pathogenic bacteria in protozoan (*Tetrahymena*) vesicles. This knowledge can be applied to predict the survival of other potential pathogens in the environment. The bacteria will be added, individually in a ratio of 100:1 cells to a starved *Tetrahymena* culture. Concentrations of bacteria and *Tetrahymena* will be enumerated every 24 hours for five days to establish the feeding preferences of the selected protozoan. In addition, different growth phases of the bacteria will be investigated. *Salmonella* spp. and *Aeromonas* spp. in different phases of growth (log and stationary) will be added to starved *Tetrahymena* to determine whether or not *Tetrahymena* displays a preferential feeding pattern. The ingestion pattern of *Salmonella* compared to *Aeromonas* could predict field relationships between predator and prey. Tetrahymenal vesicles will be analyzed in all experiments using the live/dead stain, DAPI and PI, under UV light to detect surviving bacteria within the vesicles. Preliminary data to date indicates no preference in bacterial growth phase with respect to the observed feeding pattern in *Tetrahymena*.

Dang, Edward. Department of Biology, McMaster University

Test of Hypothesis to Explain Mutations and Drug Resistance in Two Strains of *Cryptococcus*

Neoformans

Cryptococcus neoformans is an opportunistic human fungal pathogen that kills approximately 5-10% of patients with AIDS. *C. neoformans* is usually found in a haploid state that can be distinguished by their sex (MAT α or MAT α) but it is not uncommon to find some diploid strains. Recently some strains of *C. neoformans* have

developed resistance to Fluconazole, one of the leading drugs for the treatment of infections caused by *C.neoformans*. Twelve different clones at 4 different drug concentrations of two haploid strains (YZ2 and CHY620) of *C.neoformans* were obtained to select for drug resistant progeny. This selection process resulted in 2 parent populations (YZ2 and CHY620) and 48 different clones of each parent or 96 total clones. The minimal inhibitory concentration (MIC) of all 96 clones was obtained and compared to the MIC of the original parents. Then all 48 clones of one strain were mated with the original parent and the MIC was obtained for the diploid progeny. Preliminary results show that the MIC for all of the selected clones was significantly higher than that of the original parents. The dominance/recessiveness of these mutations are being assessed through genetic crosses and the analysis of haploid and diploid progeny. My results will help understand how drug resistance develops and how resistant mutations interact with each. Such information should facilitate the development of treatment and prevention strategies for fungal infection.

Dannell, Tracy A. and Schulte-Hostedde, A. Department of Zoology, Laurentian University.

Correlates of geographic variation in testis size of Walleye (*Sander vitreus*)

Sperm competition plays a major role with respect to variation in reproductive success, in many fish species. Walleye (*Sander vitreus*) are external fertilizers and sperm competition may be important in their mating system. I examined geographic variation in relative gonad mass, ejaculate investment of male walleye in 72 lakes in Ontario from an eight year data set collected by the, Ontario Ministry of Natural Resources Freshwater Ecology Unit of Sudbury. Statistical analysis was performed on the extrinsic and intrinsic variables affecting ejaculate investment. The clarity of the lakes and the nutrients present in the lake were correlated with ejaculate investment. The size and nutrient supply of a lake is in direct affect to the ejaculate investment of the male walleye. The morphoedaphic index is a principle method to represent fish productivity and was positively correlation with the gonad investment. In addition ejaculate investment was highest in populations with male-based sex ratios and the males that invested heavily in ejaculates were in poor condition. These results are consistent with respect to sperm competition and the energetic costs of ejaculate investment.

Dhekney, Kalindi, and Grant B. McClelland. Department of Biology, McMaster University.

Characterization of Spatial Red and White Muscle Remodelling in Zebrafish after Cold Acclimation and Exercise

Cold exposure in fish induces phenotypic changes in muscle similar to exercise training. Paradoxically, unlike mammals exposure to cold induces a decrease in metabolic rate in ectotherms. Previous studies using mixed muscle samples from *Danio rerio* have examined the molecular mechanisms of exercise and cold-induced metabolic remodelling. The purpose of the present study was to determine whether spatial muscle remodelling may influence mixed muscle measurements. Muscle cross-sections from exercise-trained or cold-acclimated (18°C) zebrafish were stained to differentiate between red and white muscle and were imaged using ImageJ software. Our results suggest that spatial muscle remodelling does not influence molecular mechanisms that direct changes in metabolic rates during cold-acclimation and exercise-training.

Dick, Mary Anne and Robin M. Slawson, Department of Biology, Wilfrid Laurier University.

Persistence and potential growth of *Escherichia coli* O157:H7 in aquatic environments.

Escherichia coli O157:H7 is a particularly virulent pathogen known to cause haemorrhagic colitis in human beings. As of yet there has been little research to assess the potential threat of an outbreak via a contaminated water source. The objective of this study is to examine the survival and growth of *E. coli* O157:H7 at different incubation temperatures in distinctive aquatic environments. Each water sample collected is first sterilized to remove the natural populations. Then, three 60 ml aliquots of the sample are inoculated with equal amounts of *E. coli*, *E. coli* O157:H7, and both *E. coli* and *E. coli* O157:H7, respectively. These flasks are then incubated at 10°C, 150 RPM. At 8, 16, 24, and 32 hours after inoculation, a sample from each flask is spread plated onto LB agar and incubated for 24 ± 2 hours at 37°C to determine the colony forming units (CFU). This procedure is then repeated with the same water source at 30°C before a new water source is tested. As *E. coli* is the indicator organism for water testing procedures, it is expected that *E. coli* should be cultured and have a significantly higher CFU for every water sample type and temperature that *E. coli* O157:H7 survives. Also, since it prefers a warm-blooded host, all samples should exhibit denser growth at 30°C than at 10°C. In testing how well *E. coli* O157:H7 survives in environmental aquatic systems as compared to indicator *E. coli*, potential situations where

E. coli may fail to indicate the presence of a pathogen may be identified. This information could be used to improve water testing procedures and prevent future outbreaks.

Dickie, Kira, J., and Sheffield, William. P. Dept. of Pathology and Molecular Medicine, McMaster Univ.

Domain Exchange Mutagenesis of the Serpins Heparin Cofactor II & α_1 -Proteinase Inhibitor M358R

Members of the serine protease inhibitor (serpin) superfamily regulate inflammation and coagulation. Serpins have a conserved core structure of 9 α -helices, 3 β -sheets, and a protruding reactive centre loop. Cognate proteases attack this loop, but are then trapped in an inactive covalent complex. α_1 -Proteinase Inhibitor (α_1 -PI), a serpin that ordinarily inhibits neutrophil elastase, can be converted into an effective thrombin inhibitor by the M358R mutation. Our laboratory has attempted to maximize the anti-thrombin activity of α_1 -PI-M358R for possible use as an antithrombotic agent. Another serpin, Heparin Cofactor II (HCII), contains a unique 75 residue N-terminal extension outside of the core, that provides an additional thrombin binding site. Fusing this extension to α_1 -PI-M358R produced HAPI-M358R, in which the rate of thrombin inhibition was increased 20-fold over α_1 -PI-M358R (Sutherland JS et al, J Thromb Haemost 2005; S1: P1200). HAPI-M358R consisted of: an MSH₆ “tag”; HCII residues 1-75; an ELG₆ spacer peptide; and α_1 -PI-M358R residues 1-394. Our objectives were to determine: the need for the spacer peptide; and the consequences of substituting additional portions of HCII for those of α_1 -PI in HAPI-M358R. PCR-directed mutagenesis was used to construct plasmids encoding: HCII 1-75 fused directly to α_1 -PI-M358R 1-394 (HAPI-75); HCII 1-132 fused to α_1 -PI-M358R 51-394 (HAPI-132); and HCII 1-201 fused to α_1 -PI-M358R 114-394 (HAPI-201). HAPI-75 and HAPI-132 but not HAPI-201 were abundantly expressed by *E. coli*, and were purified by nickel-chelate and ion exchange chromatography. HAPI-75 and HAPI-M358R inhibited thrombin at indistinguishable rates either in gel-based or kinetic assays. In contrast, HAPI-132 showed reduced inhibitory activity and partially purified HAPI-201 did not inhibit thrombin. We conclude that the HCII and α_1 -PI domains of HAPI-M358R fold independently without the need for a spacer, and but that internal interactions between helices A and D and corresponding structures elsewhere in α_1 -PI are required for optimal thrombin inhibition.

Dobrzyniecka, Sylwia. Department of Biology, Laurentian University.

Genetic variation in black spruce populations growing in contaminated areas in the Sudbury region using ISSR markers

The Sudbury region in Ontario, Canada is known for the mining and smelting of high sulphide ores containing nickel, copper, iron, and precious metals. Much attention has focused on the environmental effects of mining and smelter pollution. Although reports provide information of metal levels in soil and their uptake and accumulation by plants, knowledge of genetic effects on plants growing in contaminated areas is limited. Several authors have reported differences in genetic structure of plants growing in contaminated areas. The purpose of the present study was to analyze genetic variation in five black spruce populations growing in the Sudbury area. ISSR analysis of tree samples from these five sites revealed different levels of genetic variation within and among population. The genetic distance data showed that the four populations from the contaminated sites were genetically close and that they were different from the control site.

Elder, Christine E., and Jacobs, J. Roger. Department of Biology, McMaster University.

The Morphogenic Function of C-terminal Slit in the Developing Heart of *Drosophila*

In *Drosophila*, Slit plays a morphogenic role in the developing central nervous system and heart. Typically, Slit is secreted by guidepost cell sets to attract or repel cells that express Robo receptors. However, in the developing heart, Slit and Robo2 are both expressed in cardiac cells. Slit works to determine the correct migration pattern and alignment of these cells during cardiogenesis. Slit signaling through Robo requires the N-terminal portion of the Slit protein, which contains leucine-rich repeats (LRRs). Surprisingly, a loss of Slit function in the heart can be partially restored by expression of Slit lacking the LRR region. This project examines the function of Slit in the developing heart by using a construct containing only the C-terminal portion of Slit. Using the UAS/GAL4 system, C-terminal Slit was overexpressed in the heart. This overexpression revealed a mutant phenotype in which cardiac cells were misaligned and elongated. These results demonstrate that the C-terminal portion of Slit, independent of the LRR region, is important for Slit function during morphogenesis. This suggest that there exists an alternative signaling pathway from the one previously identified in the developing nervous system, known to require the LRR region of Slit for signaling through Robo2.

Elsohemy, A., Bain, J.R.², and Fahnestock, M.^{3*} Undergraduate student, Department of Psychology, Neuroscience, and Behaviour, ²Department of Psychiatry and Behavioural Neurosciences, ³Division of Plastic Surgery, Department of Surgery, McMaster University.

The Effect of Sensory Protection on the Muscle Spindle following Peripheral Nerve Injury

Major peripheral injury can result in muscle denervation. The outcome of nerve surgery is likely to be poor if reinnervation is delayed due to a long distance required for nerves to regenerate and reinnervate muscle. Prolonged muscle denervation results in irreversible muscle fiber atrophy, connective tissue hyperplasia, and muscle spindle deterioration. The protective effect of temporary sensory innervation on denervated muscle, prior to motor nerve repair, has been shown in the rat. Sensory protected muscles exhibit less fiber atrophy and connective tissue hyperplasia than denervated muscles. The goal of this study was to determine whether temporary sensory innervation can also protect muscle spindles from deterioration. Nine Lewis rats were divided into three groups: 1) unilateral transection of the tibial nerve (motor and sensory) from the gastrocnemius muscle; 2) unilateral transection of the tibial nerve with immediate repair of the peroneal nerve (motor and sensory) to the tibial nerve stump; and 3) unilateral transection of the tibial nerve with attachment of the saphenous nerve (sensory) to the tibial nerve stump ("sensory protected"). Unoperated contralateral gastrocnemius muscles served as controls. At six months postoperatively, muscles were prepared for histological analysis. They were embedded in paraffin wax and sectioned at 10 μ m. Every tenth section was stained with hematoxylin and eosin and examined using a Zeiss microscope. Muscle spindles were counted. Control (unoperated muscle) contained 24, immediate motor repair contained 18, sensory protection had 15 and denervated muscle had 6. Spindles in the denervated muscle were in the process of disintegration, whereas sensory protected spindles were relatively intact. Therefore, sensory protection partially protects spindles from deterioration. A significant preservation of spindles in sensory protected muscle would support the hypothesis that sensory nerves can exert protective effects on denervated muscle and promote the use of this strategy in improving the outcome of peripheral nerve surgery.

Esseltine, Jessica. Department of Biology, University of Western Ontario.

Development of A Murine Single-Cell Mutation Load Assay

Established mutation detection assays are limited by the inability to detect mutations *in situ* in a range of tissues. Detection of mutations *in situ* permits the investigation of single mutant cells within the architecture of a tissue and allows the determination of the mutation load (the frequency and type of mutations) for a particular cell type. A recently developed mutation detection system, **Single-Cell Immunohistochemical Mutation Load Assay (SCIMLA)**, detects mutant cells through immunohistochemical staining for an overabundance of the tumour suppressor p53 protein in the nucleus. This overabundance of p53 protein arises due to increased half-life resulting from missense mutations. SCIMLA, first developed for human tissues, has the potential to be extended to any organism using any cell type expressing endogenous p53. A murine SCIMLA assay would allow measurement of mutation load with age, mutagen exposure or onset and progression of diseases such as cancer. Toward the development of a murine SCIMLA assay, three month old male C57Bl mice were exposed to 0, 50 or 150 mg/kg of the known mutagen ENU via a single intraperitoneal injection and all tissues were harvested after a 15 day manifestation period. Two antibodies will be tested for staining cryosectioned tissue: either a fluorescently tagged p53 polyclonal primary antibody or a p53 N-terminal primary monoclonal antibody followed by a fluorescently tagged secondary antibody. The assay will be optimized using liver tissue sections and applied to other tissues, in particular tumours with likely overabundance of p53. The use of three different concentrations of the known mutagen as well as the tumour tissue permits the evaluation of the murine SCIMLA assay. Determination of mutation load *in situ* using an endogenous gene as mutational target overcomes current limitations with established transgenic assays.

Faheim, Sandy and Stone, Jonathon. Department of Biology, McMaster University.

Reinterpretation of Echinoderm homologies in light of skeletal meso1 expression and the Extraxial-Axial theory (EAT)

Assessing homologous traits in the phylum Echinodermata traditionally has been problematic due to a lack of a framework for characterizing features on the basis of, ontogeny, morphology, and paleontology. This problem has resulted in myriad interpretations of comparative anatomy, which have vexed establishing universally accepted phylogenetic relationships among classes. David and Mooi (1998) suggested a new theory according to which traditional structural homologies among echinoderm classes may be reinterpreted. The Extraxial Axial theory (EAT) provides a system that enables researchers to recharacterize echinoderms on the basis of two types of skeleton: the axial components formed according to the ocular plate rule (OPR) and the extraxial

components. This system has ramifications for assigning homologous traits between holothuroids and echinoids (sea urchins and sand dollars). In this study, a hypothesis of homology between holothuroid and echinoid primary podia was tested using the EAT. The monoclonal antibody 6a3 was used as an immunohistochemical probe to compare expression of the skeletogenic protein meso 1 during development.

Fantetti, K.N., Poling, K.R. and Higgs, D.M. Lakehead University.

The effects of Stimulus Removal on Auditory and Neuro plasticity in zebrafish (*Danio rerio*)

Applying treatments for deafness and auditory insult appropriately requires determining a developmental window during which therapies are most beneficial. Reduction of auditory inputs during development affects both central and peripheral functioning. It is hypothesized that sensory stimulation is needed for normal auditory hair cells and brain development. Preliminary data in our lab show that rearing environment affects auditory development but the timeframe of this effect is unknown. Central and peripheral structures were measured to determine if removal of auditory stimuli affects auditory development in zebrafish (*Danio rerio*). Zebrafish embryos were placed into uncrowded tanks of one of three groups; Noisy, Quiet, or Noisy Calm. The Noisy condition consisted of aerated aquaria in a normal laboratory rearing environment. Quiet embryos were reared in aquaria without aeration or filtration in a sound-resistant room. The intensity difference between the two conditions was over 30 dB. Embryos reared in the Noisy Calm condition were reared in normal laboratory rearing conditions, but without aeration. Auditory development was determined by measuring saccular hair cell counts from fish reared in the sound free environment and comparing them to saccular hair cell counts from fish raised in normal rearing conditions. Fish in the Quiet (stimulus removal) condition appear to have less hair cells than those in the Noisy condition. The influence of peripheral plasticity on the central nervous system was determined by measuring the volume of the MON (medial octovolateralis nucleus) of the zebrafish brain in fish raised under the two experimental conditions. There appears to be no difference in MON volume between treatment conditions, suggesting stimulus removal affects hair cell number but not brain morphology. Understanding central and peripheral development may provide a developmental window for treatment options for deafness and auditory insult in humans.

Flowers, Cindy D. and Martinez, Mery L. Department of Biological Sciences, Laurentian University.

Evaluation of the physiological status of wild populations of Central Mudminnow (*Umbra limi*) and Fathead Minnow (*Pimephales promelas*) from the Greater Sudbury area

In natural aquatic environments, fish are subjected to various temporal and spatial variations, which are likely to affect both the general physiology of the fish. We used fathead minnow and central mudminnow, common inhabitants of adverse environments (Silver Lake: D.O \approx 5.3 mgL⁻¹; Daisy Lake marsh: D.O \approx 1.0 mgL⁻¹) within the Greater Sudbury area. We examined haematocrit levels to determine the blood oxygen-carrying capacity and the activity of lactate dehydrogenase (LDH) to evaluate the tissue anaerobic capacity. The activity of LDH was examined in four tissues: white skeletal muscle, liver, brain, heart. The haematocrit levels were higher in fathead minnow ($p=0.035$). Our results showed a sex and tissue specific variation on the levels of LDH, liver activity in the male fathead minnow was higher ($p=0.05$) and the muscle activity in the female central mudminnow was greater ($p=0.015$). Tissue mass (g) had a major effect on the activity rates of the muscle and brain tissue in central mudminnow ($p < 0.0001$, for each tissue) and on the activity levels of the muscle in fathead minnow ($p=0.008$). Length and body mass had an important effect on all parameters measured for central mudminnow ($p < 0.03$). These results suggest changes in tissue enzyme are tissue specific, supporting the idea of different tissue metabolic demands.

Gaw, Ashley E., April Hayward, and Jurek Kolasa. Department of Biology, McMaster University.

Changes in density-mass relationship in response to ecosystem size changes in Jamaican rock pools.

We investigated whether the relation between organism density and mass changes with increasing ecosystem size in aquatic rock pool microcosm ecosystems on the north coast of Jamaica. We have previously shown that size-specific ecosystem respiration rate decreases as ecosystem size increases with a slope of -0.25. The respiration rate of each rock pool must equal the sum of the respiration rates of all the individual organisms present. Thus, there are two obvious factors that could cause the observed decrease in ecosystem respiration rate with increasing ecosystem size. (1) The density of organisms in an ecosystem may decrease as ecosystem size increases, resulting in fewer organisms utilizing energy as ecosystem size increases. We found that the density of organisms decreases a function of ecosystem size to the power of -1.0427 ($r^2 = 0.4926$, $p < 0.05$). (2) Since individual organisms' total respiration rate increases as a function of body mass to the power of 0.75, the

relation between ecosystem respiration and ecosystem size could be explained if larger organisms inhabit larger ecosystems. It has also been found that as the mass of an organism increases, the organism's density decreases to the -0.75. We found that the average mass of organisms decreases with an ecosystem (b = -0.6406, $r^2 = 0.6352$, $p < 0.05$) and that the size range of organisms in the system also decreases with increasing ecosystem size (b = -1.6124, $r^2 = 0.7462$, $p < 0.05$). Although the average mass-ecosystem size relationship shows that smaller organisms are found in larger ecosystems with an increased respiration rate, this relation is balanced by the negative density-ecosystem size relationship, resulting in decreased respiration rate in larger ecosystems overall.

Glover, Alexander W.*, Tarnopolsky, Mark A., and Devries, Michaela C. Department of Pediatrics, McMaster University.

The effect of exercise on Lean and Obese individuals in relation to Intramyocellular lipid and mitochondrial Morphology

In recent decades there has been an alarming increased prevalence of obesity in Canada. Obesity results from an excess storage of lipids occurring primarily in adipose tissue, however significant stores can exist elsewhere in the body. Elevated lipid deposits in these secondary tissues can result in cellular dysfunction and pathogenesis. An example of this lipotoxicity is evident with an increase in intramyocellular lipid (IMCL) content in skeletal muscle. Typically these cells develop insulin resistance and decreased carbohydrate (CHO) uptake and metabolism. As skeletal muscle accounts for 70-80% of plasma CHO uptake, if IMCL content is associated with severe myocellular insulin resistance, type two diabetes may also develop. Paradoxically, endurance trained athletes also show increased IMCL content but show marked insulin sensitivity compared to untrained controls. In order to determine if morphological differences in myocellular mitochondria and lipid droplets are associated with increased lipid content and exercise, we obtained muscle biopsies from sedentary, lean and obese individuals pre and post an intervention of 12 weeks of endurance training.

Hall, Simon H., and Ramsay, Scott M. Department of Biology, Wilfrid Laurier University.

Genetic correlates of hematozoon infection in white-throated sparrows

The white-throated sparrow (*Zonotrichia albicollis*) is a species suspected of having its fitness reduced by the presence of blood parasites (hematozoa). These parasites have been well studied, and the prevalence and intensity of their infections has been related to genetic factors, such as heterozygosity, in hosts such as the white-crowned sparrow (*Z. leucophrys*). A high level of heterozygosity at several loci, particularly at the major histocompatibility complex, has been correlated with an improved resistance to parasitic infection. The objective of the current project is to establish whether such a heterozygote advantage is also present in white-throated sparrows, a common migratory species that spends the summer months in eastern Canada. Blood samples were collected from 46 male sparrows breeding in Algonquin Park, Ontario. Blood smears were made and examined for the presence of blood parasites, particularly members of the *Haemoproteus* genus. Parasite loads ranged from 0 to 335 infections in the 10 000 cells examined for each bird, and 30.43% of the birds showed no signs of infection. DNA was also extracted from the blood samples and will be used to obtain microsatellite genotypes for each bird. Heterozygosity will then be related to the intensity of the parasitic infection of every individual bird. After this analysis has taken place, it will be determined if the heterozygote advantage found in the mountain white-crowned sparrow exists in the white-throated sparrow as well. The discovery of a heterozygote advantage in *Z. albicollis* would provide valuable information about how this species deals with pressure from parasitic infections, as well as suggesting that a heterozygote advantage may exist in other sparrow species.

Haq, Aamir. Department of Biology, Laurentian University.

Mitochondrial function and myopathic markers of skeletal muscle myopathy in mice lacking Bpag1 crosslinking protein

Studies have shown that the cytoskeleton is involved in the movement and localization of mitochondria. Whether this has any effect on the metabolic function of mitochondria remains unclear. Bpag1 is a crosslinking protein belonging to the plakin family of cytolinker proteins. The muscle isoform, Bpag1b, functions to maintain cytoskeletal integrity of skeletal and cardiac muscle fibers. Mice lacking Bpag1 suffer a sensory neurodegradation syndrome called *dystonia musculorum (dt)*, which ultimately leads to skeletal and cardiac myopathy. We have demonstrated that in *dt* mice, mitochondria of hind limb muscles aggregate at the periphery of myofibers rather than remaining homogeneously dispersed. We then investigated whether this abnormal

localization affected mitochondrial energy production and found that the enzymatic activity of succinate dehydrogenase in the electron-transport chain was not reduced or increased significantly. This suggests that energy production by the electron-transport chain is not affected in skeletal myofibers of *dt* mice. Common markers have been established for major myopathies such as muscular dystrophy, and these include i) increased fiber degeneration/regeneration cycles indicated by central nucleation and ii) large fiber size variability. However, no markers have been established for the *dt* condition. Therefore, our analysis of central nucleation and fiber size variability in *dt* mice revealed that they are not characteristic markers of the myopathy caused by the *dt* condition.

Hillman, Ariana S., Tobias J.-L. Doederlein, Reehan S. Mirza and Greg G. Pyle. Department of Biology, Nipissing University.

Effects of copper on the ability of fathead minnows (*Pimephales promelas*) to select a high condition mate

Organisms rely on multiple cues for mate selection and can use both chemical and visual cues to determine the underlying condition of a potential mate, thereby allowing the selection of a high condition mate and improving the chance of passing genes onto the next generation. Prior studies have indicated that under clean conditions male fathead minnows (*Pimephales promelas*) prefer water conditioned by sexually mature females over water conditioned by juveniles; however, copper pollution impairs this preference. Mate selection using either visual or chemical information to evaluate condition has not been studied previously in fathead minnows. The first objective of this study was to test whether minnows could differentiate between a high and low condition mate based on either chemical or visual cues. The second objective was to determine how copper affects this choice. Sexually mature minnows (males and females) were exposed to either 0 µg/L or 20 µg/L of copper for 48 hours and then tested behaviourally with cues from a high and low condition member of the opposite sex utilizing a two-channel maze. Differential condition was established by feeding minnows on high or low food regimes for 30 d. Fish were then tested chemically (water conditioned by the high and low condition fish) or visually (high and low conditioned minnows in sealed chambers at the end of each arm of the maze) for preference in the maze. Under clean conditions, females significantly preferred water conditioned by high condition males but exhibited no preference in the visual trials. Males however did not exhibit a preference for high or low condition females either visually or chemically. Effects of copper exposure will be discussed.

Hoggard, C.M. and MacDougall-Shackleton, E.A. Department of Biology, University of Western Ontario

Heterozygosity and repertoire size in an outbred song sparrow (*Melospiza melodia*) population

Elaborate secondary sex characteristics evolve through intersexual selection if they advertise some benefit to a potential mate. In song sparrows (*Melospiza melodia*), song repertoire size (the number of different song types a male is able to sing) is used by males to attract females. The larger the repertoire size the better success that male has at acquiring a high quality female mate. This suggests that repertoire size likely advertises some underlying benefit to the female, such as male condition or parental care capability. Recently it has been discovered that heterozygosity is positively correlated to repertoire size in an inbred (island) song sparrow population. It is unknown, however, if this finding is an artifact of an inbred population, that is, driven by a few very inbred individuals of low fitness, or whether it holds true for populations in general. To further investigate the relationship between heterozygosity and repertoire size, song was recorded and blood samples taken from a population of outbred song sparrows. For each individual male, I determined the size of his song repertoire and performed genetic analysis at four microsatellite loci. The relationship between song repertoire size and various measurements of genetic variability will be discussed.

Hoogenboom, Adriana L. and Gunn, John. Department of Biology, Cooperative Freshwater Ecology Unit, Laurentian University.

Fish Community Assessment for Kelly Lake: A Contaminant Trap for Metal and Nutrient-Rich Water Flowing From the City of Greater Sudbury

Over the past century, vast quantities of sediment, metals, organic contaminants and nutrients have been deposited in Kelly Lake (339 ha, 18 m max. depth) from upstream mining and smelting facilities, sewage disposal, creosote plants, and a multitude of other urban and industrial activities. The extensive erosion of surface soils from the once near-barren Upper Junction Creek watershed also added huge amounts of sediment that formed an extensive delta area. Kelly Lake has thus served, and still serves, as a valuable contaminant trap that has helped to protect the downstream watershed that extends 100 km to the mouth of the Spanish River entering Lake Huron. Water quality in Kelly Lake is now much improved due to large scale investments in

effluent treatment and watershed rehabilitation, although it is still severely impacted. The first quantitative fish assessment of Kelly Lake was conducted in 2005. Surveys were also conducted in three comparable upstream lakes (Ramsey Lake, Maley Reservoir - low nutrients, and Bethel Lake - high nutrients). Fish species richness was highest in: Kelly Lake - 15, (Maley Reservoir - 12, Ramsey Lake - 8, and Bethel Lake - 6). Average biomass per net was also highest in Kelly Lake - 79.8 kg/net (Bethel Lake - 28.8 kg/net, Ramsey Lake - 26.7 kg/net, and Maley Reservoir - 18.7 kg/net). Due to their presence and abundance in all four lakes, common white sucker, *Catostomus commersoni*, was used to examine variation in liver somatic index (LSI = liver wt (g) / (body wt (g) – liver wt (g)). LSI values were significantly higher in Ramsey Lake and Kelly Lake while being lower in Bethel Lake and Maley Reservoir. LSI did not correspond to nutrient levels. This initial survey revealed a relatively diverse and abundant fish community in Kelly Lake as well as significant unexplained differences in sucker LSI values between the lakes.

Hughes, Andrew. H. and Schellhorn, Herb. E. Department of Biology, McMaster University.

Chromosomally-encoded Inducible *rpoS*

σ^S , or RpoS, is an alternative σ factor in *Escherichia coli* and other γ -proteobacteria, controlling genes involved in responses to many different kinds of stress. The RpoS regulon is now known to consist of more than 130 positively regulated genes identified by random *lacZ* promoter-fusion technology, and more recently, DNA microarray technology. Chen and Schellhorn (2003) utilized the pET protein induction system to create *E. coli* MC4100 derivatives that could be controlled for the expression of *rpoS*. This allowed the expression of *rpoS* under non-physiological conditions, allowing study of RpoS function independent of other stationary-phase transcriptional regulators. Further to this work we have developed a system for integration of the T7 promoter induction system in place of the native *rpoS* promoter. This will allow the inducible control of a single copy of the *rpoS* gene without the plasmids used in the previous system. This is advantageous because it eliminates problems of multiple gene copy number, as well as the metabolic burdens of the plasmid itself and the selectable marker that it encodes. The chromosomally-encoded inducible *rpoS* will be studied first by titration of levels of induction producing varying amounts of RpoS. Future studies include microarray or other gene exp

Ibrahimi, Ali. McMaster University.

RpoS-mediated gene expression in *Escherichia coli*: a comparison of the RpoS-regulons of *E. coli* strains MG1655 and GC4468

RpoS is at the core of a sensory response system that integrates cellular and environmental stress signals and reacts with an appropriate response: it upregulates gene products that promote survival during stress and decreases transcription of genes not immediately important for the stress-coping response. Despite its profound modulation of the overall gene expression profile of *E. coli*, the RpoS regulon remains incompletely characterized (Patten *et al.*, 2004). We have examined genome-wide RpoS-dependent gene regulation in two previous studies (Vijayakumar *et al.*, 2004; Patten *et al.*, 2004).

In this study, we have measured expression of genes previously found to be RpoS-dependent in *Escherichia coli* GC4468 in *E. coli* MG1655 using *lacZ* fusions to measure B-galactosidase activity. Our results were similar to the previous study as most of these genes were, in fact, also RpoS-dependent in MG1655 at stationary phase in rich medium. We also found that both absolute expression levels and RpoS-dependence were generally higher when genes were assayed in GC4468. Furthermore, we assayed for RpoS-dependent expression at hyperphysiological RpoS levels and found that the majority of the RpoS-dependent genes were expressed at significantly higher levels at normal RpoS concentrations.

Iftikar, Fathima I., Patel, Monika., Wood, Chris M. and Ip, Alex. Dept. of Biology, McMaster University.

The Effects of feeding on Ion Regulation in African Lungfish *Protopterus annectens*.

Many studies have focused on air breathing, aestivation and biochemical analysis of related organ systems in the African lungfish *Protopterus annectens*. However, knowledge in the area of *P. annectens* nutrition is little known. Lungfish are studied as possible models of tetrapod evolution and thus, there is a need for further research regarding the nutrition they receive and their consequent physiological responses to feeding. The objectives of this study were to examine the effects of feeding on ion regulation in *P. annectens*. The regulation of ions, specifically that of Na^+ , Cl^- and Ca^{2+} , in response to a diet of blood worms in *P. annectens* was studied. Na^+ and Cl^- are essential minerals in animals because of their role in electrolyte and acid–base balance; Na^+ is the principal extracellular cation, whereas Cl^- is the principal intracellular anion in animal tissues. Fishes are known to readily exchange these minerals across their gills in order to maintain acid–base balance and osmotic

pressure with their aquatic environment. Ca^{2+} is an important nutrient to lungfishes as it is used to mineralize the toothplate of their mouth and in the development of the lungfish skeleton. It was found that *P. annectens* differentially regulate the major ions (Ca^{2+} , Na^+ and Cl^-) after feeding. They continuously uptake Ca^{2+} at an increasing rate from their aquatic surroundings possibly through the chloride cells on their gills and skin surface. Na^+ seems to be excreted to the environment perhaps via urine or feces while Cl^- is maintained throughout the feeding period.

Jackson, P.J. Faculty of Forestry and the Forest Environment, Lakehead University.

The phenomenon of woody accidental epiphytes in the canopies of urban street trees in Thunder Bay.

The occurrence of accidental woody epiphytes in the canopy of urban street trees of Thunder Bay, Ontario is presented. A plant is considered an accidental epiphyte if it is normally found as a terrestrial phanerogam but for some reason is found growing in the fissured bark, crotch or branch stub of an established tree. The epiphyte is not acting parasitically, but rather surviving and often thriving in a small accumulation of humus that has formed in the crotches of these trees. The percent of street trees in Thunder Bay with accidental woody epiphytes appears to be unusually high. Of 6251 street trees inventoried in the summer of 2005, 352 (or 5.63%) had accidental woody epiphytes growing in their canopies. Of these host trees, 203 were thoroughly surveyed during the fall of 2005. Thirty five species of street trees were encountered during the survey but only eight of them served as hosts to accidental woody epiphytes. Silver maple (*Acer saccharinum*) and paper birch (*Betula papyrifera*) hosted 95% of the observed epiphytes. The composition of street trees in Thunder Bay is dominated by ageing, and often declining silver maple and paper birch. There is also a large component of *Fraxinus* species in the city, however, these trees only hosted 0.3% of the observed epiphytes. It suggested that the architectural canopy structure of silver maple and paper birch lends itself more readily to colonization of accidental woody epiphytes than the structure of ash. Fourteen genera of accidental woody epiphytes were observed on the 203 surveyed trees. Of these, 94% were from six genera: *Sorbus*, *Betula*, *Sambucus*, *Rubus*, *Cornus*, and *Prunus*. The three most common host tree/woody epiphyte combinations were silver maple/mountain ash, silver maple/paper birch, and paper birch/mountain ash. Seed dispersal will be discussed in relation to frequency of occurrence. An insight will be provided as to why Thunder Bay has such a high rate of occurrence.

Jayasuriya, K.M. and Woodin, M.A. Department of Zoology, University of Toronto.

Developmental Regulation of GABA and KCC2 in the Zebrafish (*Danio rerio*) Visual System.

The neurotransmitter γ -aminobutyric acid (GABA) undergoes a dramatic developmental switch; it is excitatory in immature organisms while it is inhibitory in the adult. The long-term objective of our study is to determine whether activity can regulate the time in development when this switch occurs. We have chosen the visual system of Zebrafish (*Danio rerio*) as our model system for its well-characterized advantages as a molecular model system as well as its well defined visual anatomy. As a first step in addressing our long-term goal we wanted to determine the developmental expression of GABA in the retina. To do so, Zebrafish embryos at 3, 4, 6, and 9 days postfertilization (pfd) were fixed in paraformaldehyde and sectioned across the dorsal-ventral axis. GABA expression was detected by confocal microscopy in conjunction with immunocytochemistry using antisera against GABA. Our preliminary results showed a transient expression of GABA in the retinal ganglion cell layer, in which the GABA expression observed at 3pfd diminished at 4pfd and eventually disappeared by 6pfd.

The switch in the polarity of the GABA response is determined by the expression of KCC2, a neuron-specific K^+/Cl^- cotransporter, which rapidly extrudes chloride from the developing neurons switching GABAergic transmission from excitatory to inhibitory. Thus, we next asked whether the developmental expression of GABA correlated with the up-regulation of KCC2. Using Western blot analysis we demonstrated that the neuron-specific expression of KCC2 starts at around 3pfd and increases gradually until 9pfd. These results are the basis for future studies that will be carried out to examine whether sensory-input itself can regulate the GABA switch through regulation of KCC2 expression and to investigate the role of GABAergic synaptic transmission in visually guided behaviours.

Kanagaratnam, Menaka, Huntley, Melanie, and Golding, Brian. Dept. of Biology, McMaster University.

The metrics of Protein Repeats: From Simulation to Application

The term protein repeat often refers to a segment of a protein sequence that is composed of one or a few amino acids that are tandemly re-iterated. Repeats can be more broadly classified based on their length, proximity, and amino acid composition. Low-complexity repeats for instance, are relatively short segments composed of a single or a few similar amino acids. Stretches of repeat sequences may also be separated by non-repetitive sequences. It has been suggested that there are multiple means by which repeats can originate and expand. The mechanisms by which repeats arise, as well as their role in proteins, are largely unknown. It is generally believed that slipped-strand mispairing is one common mechanism. Being able to detect specific types of repeats based on a predicted mechanism for how they arose will enable researchers to classify such repeats based on their evolutionary history. Detecting these repeats, as well as defining their evolutionary history, have thus far been problematic due to the rapid changes that occur within the repeat sequences. We present a new algorithm for finding repeats within protein sequences. The algorithm is able to determine the length of the repeating fragment and the number of times it is found in a sequence. We demonstrate the ability of the algorithm to detect tandem repeats as well as non-tandem repeats that have since undergone extensive mutational change.

Kam, Joanne, Franklin, Natasha M., and Wood, Chris M. Department of Biology, McMaster University.

Diet selection in rainbow trout (*Oncorhynchus mykiss*) under chronic dietary cadmium exposure.

Cadmium (Cd) is a metal found in the environment and is produced through natural and anthropogenic processes. Chronic exposure to Cd can have detrimental effects on the calcium balance of fish through accumulation in the tissues. Supplement of Ca^{2+} through either waterborne or dietary uptake is shown to alleviate these effects, however it is not known if fish are able to consciously show food preferences that offer protection. To determine if fish have the ability to choose a diet with physiological benefits when under Cd stress, groups of rainbow trout were exposed to one of two dietary conditions in which fish were given a choice between an elevated Na^+ food (42.3 mg/g) or control food and a choice between food containing dietary Cd (500 $\mu\text{g/g}$) with standard Ca^{2+} (15mg/g) or food with dietary Cd supplemented with elevated Ca^{2+} (60mg/g). The purpose of the Na^+ trial was to show that fish are able to make a preference for a type of food. Food was dispensed through the use of demand feeders which allowed for the preference for each type of food to be measured. Strong preference for the elevated Na^+ diet was established through both the daily measurement of remaining food in each feeder and through x-raying at different time points during the exposure. Results from the Cd and Ca^{2+} exposure show no preference for the Ca^{2+} -enriched food. This may be due to the possibly unappealing taste of the Ca^{2+} -enriched food and the inadequate length of time for the chronic effects of Cd exposure to be expressed. Future work will be done exploring a protracted feeding trial with Cd and Cd supplemented with elevated Ca^{2+} diets and the food preference between control and Ca^{2+} -enriched food to determine if taste is a factor in food preference.

Keable, Lisa, and G. H. Parker. Department of Biology, Laurentian University.

Prevalence and abundance of gastrointestinal parasites in fisher (*Martes pennanti*) of Ontario, as affected by geographic location, gender and age of the host

This study constitutes one of the first reported surveys of gastrointestinal (GI) parasites in fisher (*Martes pennanti*) of Ontario and, in addition to reporting their presence and numbers, seeks to determine the extent to which both prevalence and intensity of infections vary among geographic sites and in relation to sex and age of the host species. Given the less inclement climate and the greater abundance of potential intermediate and paratenic hosts characterizing southern parts of the province, it was hypothesized that both the prevalence and intensity of infections would be less among populations in the north. Given the greater temporal opportunity for exposure with age, and greater spatial opportunity for exposure by wider-ranging males, it was predicted that infections would be more frequent and intense in adults versus juveniles and among males compared to females. Thorough examinations were conducted on the GI tracts of 159 fisher collected from three geographic regions; (a) Manitoulin Island, a southern insular site, (b) Sudbury, a local site, and (c) Timmins, a more northern site. Two species of helminthes, tentatively identified as *Physaloptera maxillaris* located in the stomach, and *Baylisascaris devosi* in the intestine, were recovered resulting in an overall prevalence of infection of 31.4%. *P. maxillaris* was present at both the Sudbury and Manitoulin sites, where it occurred at comparable frequencies and intensities, but was not found at the northern Timmins site. *B. devosi*, although not found in the Manitoulin population, was present in Sudbury and Timmins, showing prevalence values greater than 50% at the latter site. Gender differences appeared to exist only at the Manitoulin site where males indicated higher incidence and

intensities of *P. maxillaris* infection compared to females. No evidence of age-related trends were noted in the study. These patterns will be discussed in the context of the ecology of parasite-host relation

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siRNA-mediated knockdown of synapsin II in the medial prefrontal cortex of rats: implications of synapsin II in schizophrenia

Synapsin II (synII) is a neuron-specific phosphoprotein that binds presynaptic vesicles to the cytoskeleton. Previous studies have shown that the synII gene transcript is reduced in the medial prefrontal cortex (MPFC) of schizophrenic patients and that the gene as well as the protein is significantly up-regulated upon chronic antipsychotic drug treatment in rats. However, it is unclear whether the reduction in synII is a causal factor contributing to the onset of schizophrenia, or whether the reduction is a consequence of the disease process. To establish this, we investigate whether the knockdown of synII, by infusing short interfering RNA (siRNA) into the medial prefrontal cortex of rats, would result in the development of schizophrenia-like behavioural abnormalities, such as deficits in prepulse inhibition, locomotor activity, and social interaction, similar to those observed in putative animal models of schizophrenia. Preliminary studies have shown that infusing synII-targeting siRNA, for 2 weeks, significantly reduced the protein levels of synII and that the rats displayed deficits in social interaction. Further studies are being carried out to determine whether the knockdown of synII will bring about deficits in prepulse inhibition, and locomotor activity. This study is the first to show a link between reduced synII in the MPFC and the development of schizophrenia-like behavioural abnormalities in rats, implicating the role of synapsin II in the etiology of schizophrenia.

Kirwan, Tyler, Danielle Brabant, Paul Michael, and Aseem Kumar. Department of Biology and the Biomolecular Sciences Programme, Laurentian University.

Characterization of Cellular Dysfunction Pathways in Sepsis

Sepsis is the systemic response to infection and can progress to severe sepsis and septic shock. Sepsis and septic shock are the leading causes of death in intensive care units in the developed world. Approximately 800,000 people are affected with sepsis every year in the United States, and 200,000 patients will succumb to the disease. Sepsis is initiated by the presence of microbes or its components which elicits a complex signaling cascade. Consequently, there is a misregulation of proinflammatory, iNOS, cell adhesion and apoptotic responses. The host response may lead to cellular damage and eventually to multiple organ dysfunction and death. Despite the use of anti-inflammatory therapies aimed at treating the disease, there is still an increase in death rate. Therefore, studies have been initiated to better understand the pathophysiological mechanism(s) involved in mediating sepsis. We have developed a tissue culture model of sepsis which mimics the human disease. In this model we add sera derived from sepsis patients (septic sera) to human fibroblast and cardiac myocyte cell lines. Previous studies have shown that synergistic combinations of cytokines can lead to the activation of protein kinase double-stranded RNA-dependent (PKR). PKR subsequently inactivates eukaryotic initiation factor 2 α (eIF2). Inactivation of eIF2 inhibits protein translation and can cause cellular dysfunction. Therefore, PKR and eIF2 may represent therapeutic targets for modulation during sepsis.

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Characterization of the putative galactitol transport system in *Sinorhizobium meliloti*

Sinorhizobium meliloti is a gram-negative, alpha-proteobacterium that forms a symbiotic relationship with the leguminous plant species *Medicago sativa*, commonly known as alfalfa. A wide variety of sugar transport systems have been identified in *S. meliloti*, and recently a cluster of genes have been associated with the transport of a hexose sugar alcohol called galactitol. The putative galactitol operon is composed of three transport proteins that belong to the ATP-binding cassette (ABC) transporter family, and two metabolism genes. It is also speculated that other genes involved in galactitol metabolism exist outside the operon region. Reporter genes have been inserted into the genes of interest to determine the expression of the various genes in response to growth on various media. Results showed a high induction of these genes when grown on galactitol as a sole carbon source. A separate experiment was carried out to determine the location of the promoters in this operon by cloning intergenic regions into a replicating vector with a promoterless β -galactosidase (lacZ) or β -glucuronidase (gusA) gene. Promoters were identified upstream of two of the three transport genes, and it was found that a galactitol inducible promoter does not exist upstream of the putative regulator, suggesting that the regulator is transcribed with the rest of the operon. To study the effects of the regulator on the genes in the operon, a disruption of the regulator was performed by way of insertion of a spectinomycin-streptomycin

cassette. The replicating plasmids which were used to determine promoter locations were mated into the regulator knock out strain, and reporter enzyme activities were assayed. Also, a deletion of the entire putative galactitol region was made, and this strain will be used to test growth on galactitol and a variety of other media. Results from these latter two experiments are pending.

Kobylinski, Anna and Xu, Jianping. Department of Biology, McMaster University.

Using PCR to Screen for the Distribution of Unique DNA Sequences and Degradation of Nylon Oligomers by Homologous Enzymes of *Pseudomonas* sp. NK87 in a Diverse Collection of Natural Strains of the Nitrogen-Fixing Bacterium *Sinorhizobium meliloti*

Determining novel strains of DNA through genome sequencing is very beneficial as it enhances our understanding of the evolution of bacterial species such as *Sinorhizobium meliloti*. The abundance of novel DNA sequences can also provide us with the functional significance of these natural strains. ATCC 9930 is a strain of *S. meliloti* that has a genome size approximately 370 Kb larger than strain Rm1021, which has been completely sequenced, and there are several groups of sequences in the ATCC 9930 genome that are not present in the Rm1021 strain. In this experiment, PCR was used to assay the distribution of 18 random clones from strain ATCC 9930 with designed PCR primers to screen for their distribution among 59 natural strains of *S. meliloti*. Electrophoretic types (ETs) showed that natural strains of *S. meliloti* vary considerably in their genome as well as their size. Among the 59 strains, 27 share the same multilocus enzyme electrophoresis (MLEE) type, ET1, and the remaining 32 strains have a different MLEE type. One random clone, R-54, has been sequenced and it is completely unique from sequences found in Rm1021. Novel DNA strains from clone R-54 have either an 88% or 89% sequence identity with a gene on the plasmid of *Pseudomonas* sp. NK87. The next step of the experiment is to test the degradation of nylon oligomer membranes by using crude enzyme solutions from novel strains of *S. meliloti* of the novel gene R-54 since this protein shows a high homology to the 6-aminohexanoate-dimer hydrolase gene that is capable of degrading nylon oligomers. Nylon is a man-made hazardous compound and it would be extremely beneficial if a novel strain of *S. meliloti* could perform the same task of nylon degradation as a gene on the plasmid of *Pseudomonas* sp. NK87.

Lambie, Dara M., and Zhu, X.D. McMaster University.

Characterization of Human Mre11 Mutants

The Mre11/Rad50/Nbs1 (MRN) complex is a DNA repair proteins complex which plays a role in both the Homologous Recombination and Non-Homologous End Joining pathways in response to DNA damage caused by radiation, chemical agents and by internal errors in replication. The MRN complex also plays a role in S-phase checkpoint control, meiotic recombination and in telomere length regulation. The function of telomeres is to protect the integrity of chromosome ends and prevent chromosome degradation. Approximately 1-5% of cellular Mre11 is found at telomeres but its function here is not fully understood. Many mutations in the Mre11 gene generated in yeast have been shown to have a telomere shortening effect. The effect that these mutations may have at human telomeres is difficult to test in mammalian cells since Mre11 null mutants are lethal.

A fusion protein strategy was developed wherein wild type Mre11 and several mutant versions were fused to a telomeric protein, Rap1. The fusion proteins were then expressed in human cell lines and it was demonstrated that the proteins localized to the nucleus of the cell and co-localized with telomere specific proteins at the telomeres. Further tests indicated that these constructs were functional in DNA repair and still bound Rad50 and Nbs1 in the MRN complex. Continuing studies will examine the effect that the mutant Mre11 proteins play in human telomere length regulation

Lavery, Jennifer. Department of Biology, McMaster University.

An Investigation into the Relationship between Species Richness and Productivity

The significant loss of biodiversity over the last decade has incited much research into the factors which affect species richness (S). The relationship between productivity and species richness has become a highly debated topic in ecology. While many studies have found the relationship to be unimodal, others have found the relationship to be positive linear. Chase and Leibold proposed that these discrepancies can be resolved by considering the spatial scale: at a lower spatial scale the relationship would be unimodal, however as the scale is increased the relationship would become positive linear. To test this hypothesis I used data from 49 natural aquatic microcosms and their invertebrate fauna. Various indicators of productivity (total invertebrate density, chlorophyll concentrations) were used to compare the S found within these ponds. The data was reviewed at a local scale (among pools) and at a regional scale (aggregated data from pools on different rocks). I found a

unimodal relationship at both the local and regional spatial scale. According to theory, at the regional scale an increase in richness in ponds with high productivity should be associated with an increase in compositional differences among ponds. To test this I used Whittaker's measure of beta diversity to compare pond-to-pond species compositions. The results did not show a relationship between increased productivity and beta diversity. In conclusion, my results do not support the hypothesis that spatial scale affects the relationship between species richness and productivity. Further research is needed to evaluate the factors underlying the unimodal relationship including that habitat variability and community stability.

Leonard, Erin M., Bucking, Carol and Wood, Chris M. Department of Biology, McMaster University.

Characterization of dietary nickel uptake in rainbow trout.

The characterization of dietary Nickel (Ni) uptake through the gastrointestinal tract of rainbow trout (*Oncorhynchus mykiss*) was determined using *in vivo* and *in vitro* experimental techniques. To date, Ni transport via waterborne exposure has been well characterized in the gills, kidneys and plasma of rainbow trout; however transport of Ni via dietary exposure does not have supporting documentation. The objective of the study was to determine the mechanism and kinetic nature of Ni transport across the gastrointestinal (GI) tract. Ni is found in bodies of water heavily impacted by mining, industrial activities, weathering and erosion at a concentration reaching the limits of 4490 µg Ni/g dry weight. It appears that Ni diffuses through the GI tract via a carrier mediated ion channel up to a concentration of 30µM. Exceeding this concentration allows passive Ni diffusion through the epithelium. Although the mechanism of Ni transport across the GI tract is unclear, several studies have shown the binding competition between Mg²⁺ and Ni using the DMT1 transporter in human Caco-2 cells. As well, Ca²⁺ and Ni competition binding experiments in the gills of rainbow trout have been outlined. Therefore, using the 30µM concentration, competition binding analysis of Mg²⁺ or Ca²⁺ along with Ni will be determined to characterize Ni transport in the GI tract of rainbow trout.

Lewis, K.A., D.M. McKay, J. Lu and A. Wang. Department of Pathology and Molecular Medicine, McMaster University.

Epithelia Under Stress and the Role of TNF-α on Jeopardizing the Enteric Barrier

Hallmark characteristics of Crohn's Disease are high serum titers of tumor necrosis factor-alpha (TNFα) and susceptibility to uncoupling of oxidative phosphorylation resulting in misshapen and swollen mitochondria. The later can cause an increase in the transcytosis of commensal bacteria across epithelial layers and in tight junction permeability (controls the paracellular permeation pathway between cells). However, the effects of TNFα on metabolically stressed enterocytes are not known. Thus, the aims of this current study are: 1) to assess bacterial translocation and epithelial permeability in model epithelia exposed to non-pathogenic, non-invasive *Escherichia coli* (*E. coli*) in the presence of a metabolic stressor and, 2) to investigate if any change in epithelial permeability is augmented or suppressed by co-treatment with TNFα. Confluent monolayers of the human colon-derived T84 epithelial cell line, grown on porous filters, were exposed to *E. coli* (strain HB101; 10⁶ cfu) ± the chemical stressor 2,4-dinitrophenol (DNP; 0.1mM) (uncouples oxidative phosphorylation) ± human recombinant TNFα (10ng/ml) and transepithelial resistance (TER; indicates paracellular permeability) and bacterial translocation were assessed over a 24h period. Addition of TNFα along with *E. coli* resulted in a drop in TER and significant bacterial transcytosis. Similarly the epithelial barrier defect evoked by *E. coli* + DNP was exaggerated by the presence of TNFα. Pharmacological inhibitor studies suggest that the intracellular signaling molecule, NF-κB, is critical for the TNFα enhancement of the epithelial barrier defect. We suggest that epithelia under metabolic stress become leaky and that the subsequent movement of luminal bacteria/bacterial products across the epithelium would stimulate immune cells in the mucosa to release TNFα, which has the potential to feedback unto the epithelium to exaggerate the epithelial barrier defect and contribute to enteric pathophysiology and the induction of disease. [Funded by the Crohn's and Colitis Foundation of Canada]

Lissina, Elena and Bostan, Vadim. Department of Chemistry and Biology, Ryerson University.

The impact of two fluoroquinolones on aquatic microbial communities

The release of pharmaceutical products in natural environments is a growing concern for the quality of aquatic systems. Norfloxacin and ciprofloxacin are two fluoroquinolone antibiotic drugs recorded by several studies in water and sediment. Their main sources in the environment are sewage effluents and biosolids amending agricultural fields. We investigated the impact of norfloxacin and ciprofloxacin on microbial communities from sediment and water column in laboratory microcosms. The microcosms contained lake sediment with their

indigenous microbial community collected from northern Ontario, reconstituted pond water, *Daphnia magna*, *Hyalella azteca*, and *Raphidocelis subcapitata*. The phenotypic fingerprint of the microbial communities in the sediment was tested using Ecoplate™ Biolog® microplates. In addition, total counts of microorganisms, TOC, DOC, Chlorophyll, and identification of the dominant phytoplankton species were performed. PCA on Ecoplate results showed that both the sediment and water microbial community structures were altered due to the addition of ciprofloxacin and norfloxacin respectively. An overall inhibition of metabolic activity was also recorded in the presence of the fluoroquinolones when compared to the reference microcosms.

Luxemburger, Julia and Albrecht I. Schulte-Hostedde. Department of Biology, Laurentian University.

Ectoparasitic effects on hematocrit levels in red squirrels

The focus of this study was to investigate the parasite-host relationships focusing on the hematocrit levels of the host. Parasites are a great cost to their hosts. The host must provide enough energy to create an adequate amount of blood to feed the ectoparasites, as well as itself. Lower blood levels not only lower the blood oxygen levels, but also lower the hematocrit levels of the host. The study was conducted from May to August in the mixed coniferous-deciduous forest of Algonquin Park. When the data was analyzed using a t-test, significant differences were shown between the two anticoagulants, Heparin and EDTA, therefore the two anticoagulants were kept separate. We performed a non-parametric ANOVA to determine that there was no significance between age and sex of the host. Multiple regression was used to examine the hematocrit levels and parasite load by month, however no significance was shown. Ultimately, further studies must be conducted between ectoparasite load and host hematocrit levels to understand this relationship fully.

MacDuff, K. and Fahnestock M. Department of Biology, McMaster University.

The Biological Activity of the Pro Form of Neurotrophin-3

Neurotrophins are soluble polypeptide growth factors that are important for the development, differentiation and maintenance of neurons in the central and peripheral nervous system. The neurotrophin family includes nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5. Neurotrophins are initially generated as proproteins and can be cleaved to become mature neurotrophins. The proforms of neurotrophins are found in the human brain. The biological activity of the proforms of neurotrophins is controversial. The studies of the proforms of NGF and BDNF are conflicting as to whether the proteins are neurotrophic or apoptotic. The purpose of this study is to determine whether proNT-3 exhibits neurotrophic or apoptotic activity. A cleavage resistant form of NT-3 was created by site directed mutagenesis to alter one amino acid at the cleavage site of the NT-3 molecule (R-1G NT-3). The R-1G gene and the Wild type (Wt) NT-3 gene were subcloned into a transfer vector and co-transfected along with linearized baculovirus DNA into insect cells, producing recombinant baculovirus. These viruses were amplified and titered. R-1G NT-3, the positive control Wt NT-3, and Wt baculovirus (empty virus without the NT-3 gene) to be used as a negative control were produced in insect cell conditioned medium. These protein expressions were examined by western blot analysis for the correct product size and then quantified by ELISA. The conditioned media were concentrated in order to obtain high concentrations of the desired proteins. A TrkC activation assay and a neurite outgrowth assay using cultured neurons will be performed to determine the biological activity of proNT-3.

Maciejewski, Marcelina and Kolasa, Jurek. Department of Biology, McMaster University.

Community Assembly: Factors Characteristic of Single versus Multiple Stable Equilibria.

Community structure depends on both biotic and abiotic factors. Whether community assembly results in single equilibrium or multiple equilibria states is contested. Chase hypothesizes that low productivity, high rates of habitat connectivity and high disturbance are habitat characteristics conducive to emergence of single stable equilibria. Opposite characteristics of habitat may produce multiple stable equilibria, which are indicated by high variance in community composition. I test this hypothesis using my study community; forty nine natural rock pools on the northern coast of Jamaica. Water samples were taken annually over the course of 14 years. The composition of zooplankton (ostracods, copepods, cladocerans), worms and aquatic insect larvae were counted and segregated according to species, ensuring no greater than five percent error. I assembled the 49 rock pools into 10 and 12 groups based on either similarities in physical properties or spatial proximity, respectively, using cluster analyses (Statistica). I used PCOrd to compute Bray-Curtis similarities within the groups of pools. I found that the relationship between productivity and similarity among groups of pools of different productivity were non-significant (regression analyses: Statistica, TableCurve 2D), which means that

variance in community composition did not increase with productivity as proposed. However, when the impact of perturbation on abundance was removed, the groups clustered according to spatial proximity showed a theoretical relationship indicating multiple equilibrium states. Furthermore, the high variance and lack of significance in the relationship between similarity and productivity may indicate existence of both single and multiple equilibrium states.

MacGillivray, M.^{1,2,*} and **Szczyglowski, K.**^{1,2} ¹Agriculture and AgriFood Canada, SCPFRC. ²University of Western Ontario, Department of Biology.

LjB13-B, a Symbiotic Mutant that Specifies a Novel Allele of *Lotus japonicus* SYMRK

Legumes, such as *Lotus japonicus* have a unique ability to efficiently obtain both nitrogen and phosphorus by forming symbiotic relationships with *Mesorhizobium loti* bacteria and arbuscular mycorrhizal fungi, respectively. The response to the bacteria is to form root nodules, where air-derived nitrogen is reduced to ammonium. This renewable resource of essential macronutrients has many agricultural implications. Recent development of genetic and genomic tools in model legumes provides a solid basis for the comprehensive molecular dissection of the mechanisms underlying the nitrogen fixing symbiosis. Based on the collection of mutants developed in the model legume *L. japonicus*, the symbiotic phenotype of one particular mutant, called LjB13-B was evaluated in detail using a forward genetics approach. LjB13-B has a unique phenotype in that it retains the ability to form nitrogen fixing nodules, although the nodulation is significantly delayed and the overall number of nodules formed is reduced as compared to the parental line. On the other hand, LjB13-B is unable to interact with mycorrhizal fungi (Myc⁻). The phenotype of LjB13-B (Nod⁺Myc⁻) suggests that the affected locus serves an important function in both rhizobial and arbuscular mycorrhizal symbioses. Consequently, the locus is predicted to be an element of the common symbiosis pathway in *L. japonicus*. The *LjB13-B* locus was mapped to chromosome 2, in the vicinity of the Symbiosis Receptor Kinase (*SYMRK*) locus. The symbiotic phenotype of the LjB13-B line appears to be different from all known *symRK* mutant alleles (Nod⁻ Myc⁻). However, sequencing data confirms that LjB13-B is a novel allele of *symRK*. The results of this project are expected to extend the knowledge of mechanisms used by legume plants by allowing further characterization of *SYMRK* function during both rhizobial and arbuscular mycorrhizal symbioses.

Mahoney, Tim and **Chow-Fraser, Pat.** McMaster University, Biology Department.

Comparison of the relationship between the Water Quality Index and the Wetland Zooplankton Index, Wetland Macrophyte Index, and Wetland Fish Index for assessing human-induced disturbance in coastal wetlands of the Laurentian Great Lakes.

Several ecological indices have been developed to assess the quality of wetlands in the Laurentian Great Lakes. One of the most widely applied is the Water Quality Index (WQI; Chow-Fraser 2006), which measures the degree of water-quality impairment resulting from nutrient enrichment and road runoff that attends land-use alteration and shoreline-modification of wetlands. The WQI is based on 12 water-quality parameters, and requires the use of specialized and expensive equipment and many hours of sample processing. Therefore, other indices that rely on the type of organisms living in wetlands have also been developed because these are less labour-intensive to carry out on a routine basis, and in some cases, can be calculated within a day of sampling. Currently, the Wetland Fish Index (WFI; Seilheimer and Chow-Fraser 2006), Wetland Zooplankton Index (WZI; Loughheed and Chow-Fraser 2002), and the Wetland Macrophyte Index (WMI; Croft and Chow-Fraser, unpub. data), are all based on statistically-derived changes in biotic communities along a gradient of deteriorating water quality. In this study, we compare the performance of each of the organism-based indices against the WQI for 33 Great Lakes wetlands. The three biotic indices varied in their response to changes in water-quality conditions, and these differences were then examined through a critical analysis of the individual taxa in the three trophic levels across the degradation gradient. The overall set of interactions among fish, zooplankton, aquatic plants and water chemistry was used to explain the strengths and weaknesses of each index. Results of this study provides a basis for assessing the suitability of these indices as substitutes for the WQI.

Matthews, B., Linley, D., Lippert, K., Morgan, G. and C. Ramcharan. Laurentian University.

Variation in yellow perch gill morphology caused by piscivore introduction in Sudbury area lakes

Many Sudbury area lakes lost piscivorous fishes (e.g. walleye, lake trout, bass) due to acidification and metal contamination but retained planktivorous fishes, especially yellow perch (*Perca flavescens*). The piscivores have now returned to some recovering lakes. Thus for yellow perch, the danger of predation varies widely among lakes. In low-piscivore lakes, perch should form large, competition-limited populations that would be able to roam the open waters to consume small zooplankton prey. In high-piscivore lakes, perch should seek refuge in the littoral and benthic zones where available prey would be large invertebrates. This predator-mediated difference in perch diet should be evidenced by a change in feeding apparatus. Gill raker morphology is known to vary with fish diet, becoming fine and closely spaced when small prey are the primary forage, and becoming stout and widely spaced with large prey. We tested this hypothesis using collections (summer 2005) of forty perch (100 - 120 mm TL) from lakes without piscivores (Lohi, Hannah, and Wavy), and lakes with recently introduced piscivores (Clearwater and Middle). We took a variety of meristics from digital photographs of the outermost, left-side gill arch. In lakes without piscivores, perch had smaller gill arches (both upper and lower) with more and finer gill rakers that were more closely spaced compared to perch from lakes without piscivores. Differences were also seen in the gill raker width of the perch between the two sexes, with the females having wider rakers than the males in the presence of predators. Average gill raker length did not differ between lake types. Our results suggest that fish from piscivore-free lakes are better suited to feed on zooplankton while those from lakes with piscivores are adapted to feed on benthic macro-invertebrates. This difference in feeding morphology was supported by diet data collected from these two types of lakes.

McIntyre, Bonnie L. Department of Biology, Wilfrid Laurier University.

Purification of uncoupling proteins, expressed in *Escherichia coli*, and conformational analyses

Uncoupling proteins (UCPs) are integral inner membrane proteins of mitochondria that uncouple oxidative phosphorylation by mediating proton leak from the inter membrane space to the matrix. UCP1, found exclusively in the mitochondria of brown adipose tissue, uncouples oxidative phosphorylation in non-shivering thermogenesis. This type of thermogenesis, which occurs in many animals and new-born humans, uses oxygen and reducing equivalents to produce heat without the subsequent production of ATP. The function of UCP2, which is ubiquitously distributed in several mammalian tissues at low concentrations, is largely unknown but it has been suggested that it mediates proton leak to decrease the production of superoxide, a deleterious reactive oxygen species. The exact 3-dimensional structure of UCP proteins is unknown; thus, this study aims to determine structural information for UCP1, whose main function is largely understood, and UCP2 in order to gain insight into its function. Mouse UCP1 and human UCP2 were expressed as inclusion bodies in *Escherichia coli* strain BL21. The inclusion bodies were extracted using lysozyme and sonication, followed by centrifugation, and then purified by washing with the detergent Triton X-100. Once the proteins were ~75% pure, as determined by analyzing the intensity of their bands on SDS-PAGE gels relative to the whole insoluble protein fraction, they were solubilized in sarcosyl, which was replaced by the milder detergent, digitonin, to facilitate protein refolding. Circular dichroism spectroscopy (CD) was used to determine secondary-structural information in membrane-mimicking environments, and indicated that UCP1 was more than 90% α -helical. Current CD data for UCP2 will also be presented. It is hoped that this study will provide insight into the 3-dimensional structure of these widely distributed, yet largely mysterious proteins.

Mihic, Anton, Xiaodong Gao, Herbert Y. Gaisano, Robert G. Tsushima. Departments of Physiology and Medicine, University of Toronto.

Direct Modulation of HERG Channel Amplitude and Gating by Syntaxin-1A

The SNARE protein, syntaxin-1A (STX1A), is involved in cellular trafficking and regulation of ion channels. We have reported the expression of STX1A in cardiac myocytes, and its modulation on the expression and gating of two cardiac K^+ channels, K_{ATP} and $K_v4.2$. Thus, we examined the effects of STX1A on another important cardiac voltage-gated K^+ channel, HERG (human ether à go go), expressed in tsA 201 cells. The channel is involved in repolarization of the cardiac action potential, and HERG deregulation can lead to the development of lethal arrhythmias. Pulldown assays demonstrated HERG binds to GST-STX1A but not GST. Whole-cell patch clamp experiments demonstrated STX1A reduced peak HERG currents (at +20 mV) from 136.1 ± 7.6 pA/pF (HERG, n=18) to 42.0 ± 6.1 pA/pF (HERG + STX1A, n=16) ($p < 0.001$), and inhibited the development of C-type inactivation. There was a significant reduction in the peak outward tail current at +60 mV from 99.1 ± 8.8 pA/pF (HERG, n=18) to 43.7 ± 8.6 pA/pF (HERG + STX1A, n=16), with no shift in the midpoint of the voltage dependence of activation (-9.1 ± 1.1 mV vs. -9.4 ± 1.9 mV). We next examined the effects

of STX1A on HERG channel gating kinetics. STX1A accelerated the rate of activation between -10 mV and +60 mV, from 1908.4 ± 195.2 ms (HERG, n=16) to 1167 ± 110.6 ms (HERG + STX1A, n=10) and 45.8 ± 12.6 ms to 3.4 ± 0.7 ms, respectively ($p < 0.05$). Additionally, STX1A induced a hyperpolarizing shift in the midpoint of the voltage dependence of steady-state inactivation from -29.9 ± 4.1 mV (HERG, n=12) to -51.5 ± 4.8 mV (HERG + STX1A, n=6) ($p < 0.01$) but did not change the slope factor (20.6 ± 1.0 mV vs. 19.2 ± 1.2 mV). No change in the voltage-dependent kinetics of deactivation was observed. We have shown that syntaxin-1A may be an important intrinsic regulator of HERG channel amplitude and gating, and may have implications for LQT-related syndromes in the heart.

Supported by HSFO

Mogilnicka, Monika and Rama S. Singh. Department of Biology, McMaster University.

Investing sexual harassment and sexual antagonism in *Drosophila melanogaster*

When males and females interact during mating there arises a conflict between their different reproductive strategies. This sexual conflict will force each sex to evolve traits that allow the individual to maintain their optimal mating strategy and counter the strategy of their partner, that is males will evolve traits that allow them to persist in continuous mating which will drive females to adapt and evolve resistance traits resulting in an arms race known as Sexually Antagonistic Coevolution. Previous studies observed this phenomenon for different traits in *Drosophila* such as seminal proteins (Acps) which increase remating rate but can also be deleterious to the female. When exposed to females resistant to Acps, males evolved more toxic seminal fluid increasing female mortality. However, in case of experimentally enforced monogamy, male toxicity decreased. In the present study, we tested the effects of male density on both female survival and fecundity in the absence of selection, as well as tried to test for an optimum male to female mating ratio. The process was also reversed using males as the subject sex. Increased male density seems to have a negative effect on both female survival and fecundity, with the optimal male to female ratio being approximately 3:1. Female density seems to have no effect on male survival and male fecundity seems to increase with availability of mates. From our results we see a strong opposite effect between females and males for fecundity in relation to the number of mating partners. Moreover, even in absence of selection for a higher toxicity of seminal proteins, a deleterious effect is observed on female survival.

Morse, Andrew. Department of Biology, Wilfrid Laurier University.

Anatomy of the aborted pea nodule of R50 (a pea mutant)

Pea (*Pisum sativum* L.) is an important agricultural crop. Value is placed on pea because of its ability to grow in nitrogen-poor soils through its symbiotic association with nitrogen-fixing bacteria. In wild-type pea (Sparkle), this symbiosis occurs within nodules on the roots of infected plants. In our lab we study many pea mutants; one of which we are very interested in is R50. It accumulates cytokinin, has a short stature and an irregular root vasculature. Nodules of R50 tend to be small, white, and ineffective compared to those of Sparkle. Cytokinins are known to affect the development of vasculature, therefore the vascular structure of R50 nodules may differ in comparison to that of Sparkle nodules, possibly causing some of the mutant's characteristics. In this study, I will compare the anatomical structure of R50 and Sparkle nodules, with a special emphasis on the branching of the nodule vascular tissue from that of the root. Nodules of Sparkle and R50 were harvested after 21 days of growth, fixed in glutaraldehyde, and embedded in LR White[®] resin. Embedded nodules were then sectioned using an ultramicrotome, stained with toluidine blue O, and compared for differences in vascular structure under a light microscope. Through comparisons of the vascular structures of Sparkle and R50 nodules, I hope to determine the anatomical differences between the two, and distinguish if the development of vascular tissue is hindered in the mutant. Differences in the vasculature connecting nodules to roots could prevent nitrogen or plant products from being transported between the two symbionts, thus negating the benefits of the symbiosis.

Mucciarelli, Valerie. Department of Biology, University of Western Ontario.

Effects of veiling light on nuptial signal characteristics of threespine stickleback in British Columbia Lakes

Communication involves the generation, transmission, and reception of signals and is essential for a variety of functions including mating. Effective mating signals are those that are conspicuous with respect to the communicator's background and that transmit well through the signaling medium. Male threespine stickleback (*Gasterosteus aculeatus*) develop a nuptial signal (red chin) that is an effective signal in most coastal and

freshwater habitats. Never the less, spectral properties of water vary among different environments and consequently variation in signals may arise among populations to maintain signal effectiveness. I investigated the relationship between the veiling effect (light between the observer and an object is scattered, decreases the contrast between an object and its background) among several British Columbia lakes and the threespine stickleback nuptial signal variation. First, I developed and tested a mathematical model of signal contrast degradation based on the human visual system. Second, I applied the model to the threespine stickleback visual system to predict variability among lakes in male signal characteristics. I found that peak reflectance of male nuptial signals shifted to longer wavelengths in lakes that had the poorest transmission of red light.

Murphy, Shidan C. and Morton, Eugene. Department of Biology, York University.

Sex Ratio, Protandry and Male Size Dimorphism in a Little Known Gregarious Parasitoid: *Gambrus amoenus*

Little is known about the gregarious ichneumon parasitoid *Gambrus amoenus* (Gravenhorst) (= *nuncioides* (Townes)). This specialist parasitoid possesses interesting behaviour traits that have been previously undescribed for the species. *G. amoenus* has a highly male biased sex ratio, ranging from 15.5 to 2.5 males for every female in the population, over the four years studied. Males observe protandry both in the population and in their natal broods, avoiding Local Mate Competition, LMC. Male size was highly variable in the population, while female size was uniform. Male weight, a representation of male size, was measured by dividing the cumulative weight of the males in the brood, by the number of males in that brood. In all male broods, the chief determining factor of male weight is the number of other males present. We speculate that exploitative competition reduces average male size. Allozyme electrophoresis, used to assay for allelic variation, revealed an observed heterozygosity of zero; suggesting decreased genetic variance in the population. The investigation of these basic traits of *G. amoenus* further expands our knowledge of the poorly studied ichneumonidae.

Murray, Hollydawn and Macfie, Sheila M. Department of Biology, University of Western Ontario.

The localization of cadmium and zinc in soybean (*Glycine max* L.) using the Sulphide Silver Method.

Information on the distribution of metals in a plant provides clues to the mechanisms of metal tolerance. For example, one can distinguish between a plant that prevents the uptake of metals from the soil from a plant that tolerates increased concentrations of metals in their tissues. While much is known about the distributions of cadmium and zinc at the tissue level, information at the cellular level is scarce. At this scale, one can determine whether metals are being sequestered in sites removed from metabolic activity (e.g. the cell wall or vacuole) or whether they are present in the cytoplasm, presumably in a non-toxic form. The localization of cadmium and zinc in soybean (*Glycine max* L.) at the cellular level was determined using the Silver Sulphide Method. With this method, histological sections of plant tissues are examined using a microscope. Three treatments (cadmium only, zinc only, and cadmium and zinc) were applied to soybean plants at concentrations of cadmium (0, 50 or 100 mg/kg of soil) or zinc (0, 2000, or 4000mg/kg) or a combination of both metals (0 Cd/0 Zn, 50 Cd/2000 Zn, 100 Cd/4000 Zn mg/kg). These two metals were studied because we wished to examine the effect of one metal on the uptake and localization of the other metal. Tissues observed include roots, nodules, beans, and leaves. This work was supplemented by determining the total concentration of cadmium and zinc accumulated in bulk plant tissues.

O'Reilly, Lindzie and Chow-Fraser, P. Biology Department, McMaster University.

An investigation of abiotic factors influencing submersed macrophyte colonization in Georgian Bay coastal marshes

Nineteen wetlands in Georgian Bay and the North Channel were visited in the summer of 2005 to investigate abiotic factors influencing submersed macrophyte colonization. Since submersed wetland plants are in physical contact with both the sediment and water, we hypothesized that both soil quality (i.e. the organic, inorganic, and nutrient content of the sediment) as well as factors controlling light penetration (e.g. wind and wave disturbance, water turbidity) will influence the ability of plants to colonize an area. For sites $\leq 1\text{m}$ in depth, the amount of sediment exchangeable ammonia and the proportion of silt, clay and organic matter within the sediment were positively correlated with plant species richness while a negative relationship was observed between species richness and the proportion of sand. Absence of plants in deeper sites could not be explained on the basis of sediment quality, but is probably related to light limitation, as very little light from the surface actually reached the bottom. Results of this study suggest that an inability of submersed plants to colonize shallow areas is attributable to unsuitable sediment characteristics, whereas plant colonization in deep sites is

limited by the penetration of light through the water column. Furthermore, decreased plant species richness was found to be associated with increased levels of disturbance from human activities (e.g. boat traffic) and wind action, while more sensitive macrophyte species were found to exist in less disturbed sites. This suggests that plant species richness in a given area is not only related to sediment characteristics inherent at the site but also to the degree of human and natural disturbance experienced by a wetland.

Patel, Barkha P., Hamadeh, M.J., Raha, M., and M.A Tarnopolsky, Departments of Biology, Pediatrics and Medicine, McMaster University.

Long-term caloric restriction increases malondialdehyde in the red and white gastrocnemius muscle in the Cu/Zn-SOD mutant G93A mouse, an animal model of ALS

We previously reported that long-term caloric restriction (CR) improves motor performance but hastens clinical onset, disease progression and endpoint in the G93A mouse, an animal model of amyotrophic lateral sclerosis (ALS) (*Hamadeh et al, Muscle Nerve 2005;31:214-20; Hamadeh and Tarnopolsky, Ann Nutr Metab 2005;49 (Suppl 1):164*). We hypothesized that CR would increase oxidative stress, basal levels of which are heightened in this animal model. To test our hypothesis, we measured malondialdehyde (MDA) concentration, a measure of oxidative lipid damage, in red and white *gastrocnemius* muscle. Starting at age 40 d, 28 separately-caged G93A mice were randomly divided into two groups: ad libitum (AL, n = 14; 7 females) and CR (n = 14; 7 females), with a diet equal to 60% of AL. At age 99 d, red and white *gastrocnemius* was collected and MDA was determined spectrophotometrically at 586 nm. A 2-way ANOVA was used to determine significance, the factors being diet and sex. For the red *gastrocnemius*, MDA was higher in CR vs. AL mice (2 fold, $P < 0.001$) and in females vs. males (51%, $P = 0.047$). For the white *gastrocnemius*, CR increased MDA by 31% (mean \pm SEM: AL, 95 ± 6 vs. CR, 124 ± 17 nmol/g wet weight, $P = 0.064$), with no sex differences. We conclude that CR increases lipid peroxidation in the *gastrocnemius* of G93A mice, with females having higher values than males in the red muscle. Extrapolating our results to humans, a CR diet equivalent to 60% AL is not recommended in patients with ALS. (Funded by the Hamilton Health Sciences, HHS Foundation New Investigator Fund and NSERC Canada).

Pepper, Alicia N. Department of Biology, Wilfrid Laurier University.

Cloning and Sequencing of Cytokinin Oxidase/Dehydrogenase in Pea (*Pisum sativum* L.)

Cytokinins are phytohormones implicated in various processes of plant growth and development. The cytokinin oxidase/dehydrogenase (CKX) multigene family of enzymes is responsible for the irreversible inactivation of cytokinins. Several CKX homologs have been identified and sequenced in various plant species. The purpose of this research project is to clone and sequence the CKX gene in pea (*Pisum sativum* L.) because it is an important agricultural legume and recent evidence has linked cytokinins to nodulation. Total RNA extracted from the shoots of 17-day-old pea (cv. Sparkle) was the source of *PsCKX* template. RACE-PCR (rapid amplification of cDNA ends polymerase chain reaction), using Invitrogen's GeneRacer™ kit, was chosen as the method for amplifying the complete gene. Gene-specific primers were designed from a small, recently sequenced fragment of *PsCKX1*. These primers were used to amplify separately the 5' and 3' cDNA ends. PCR products were gel-purified and blunt-end ligated into the vector pCR®4Blunt-TOPO®. Transformed *E. coli* colonies were selected and overnight cultures were used for a miniprep plasmid isolation. Plasmid DNA containing the separate 5' and 3' fragments of *PsCKX1* were sequenced. Sequence analysis of the 5' fragment revealed that the isolated DNA was that of a cytokinin oxidase/dehydrogenase gene. The translated 5' sequence of *PsCKX1* has an FAD binding domain and shares significant sequence homology with CKX genes from *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* and *Hordeum vulgare*. Additional work must be done to obtain the sequence of the 3' end of the gene because initial sequencing was not successful. Obtaining the full-length sequence will facilitate future studies involving transcript and protein localization and will provide insight into pea development. This would be especially useful in the study of R50, a pea nodulation mutant, whose altered phenotypic traits are in part linked to an accumulation of cytokinins and altered CKX expression.

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Life on snowball Earth: a study of psychrophiles, psychrotrophs and the pigment strategies of high-Arctic bacteria.

Recent forays in space exploration and the search for life on other planets have garnered renewed interest from both the public and scientific communities. With missions to Mars and planned missions to the moons of

Jupiter, research about extremophilic organisms has become highly relevant as scientists try to determine the boundaries within which life can survive. Whether in deep oceans at extreme temperatures and pressures or in hypersaline lakes and in rock strata, organisms are found to inhabit almost every niche on Earth. Of particular interest herein are psychrophilic, or cold loving, organisms. Geologic evidence suggests the occurrence of a global glaciation, or “Snowball Earth,” event in the Neoproterozoic, with consistent temperatures below -50°C for several millennia. Although this event likely placed severe selectional pressure on the organisms of that time, it is unlikely that prokaryotes, and even eukaryotes, would not have survived such an event. Indeed, organisms have been found on the surface of ice shelves, living on the snow, in perennially ice-covered lakes and even dormant in ice-cores. To persist through such conditions, organisms would had to have acquired adaptations to extreme cold and high levels of UV radiation due to a still-forming ozone layer. To test the extent to which psychrophilic organisms could survive through similar conditions, samples of bacteria were collected from Bylot Island and Ward Hunt Island in Nunavut, Canada, for experimentation. A growth experiment was conducted on filamentous cyanobacteria and two forms of heterotrophic bacteria. An optimal growth range of 15°C was observed, suggestive of cold-tolerant organisms rather than cold-loving. Further research currently in progress will test the ability of these organisms to withstand long periods of darkness and extreme cold, mimicking conditions found in the high-Arctic. Additional experiments to assess the effects of UVB radiation on the pigment composition of these bacteria also are in progress.

Poo, Cherise and Cameron, R.K. Department of Biology, McMaster University.

Identification of Stress-Induced ARR-like Mutants in *Arabidopsis thaliana*.

Age-related resistance (ARR) refers to the relationship between a plant’s developmental state and disease resistance in some plant-pathogen systems. As *Arabidopsis thaliana* matures, it becomes more resistant to normally virulent strains of the bacteria *Pseudomonas syringae* pv. *tomato* (*Pst*), as well as to *Peronospora parasitica* (fungus-like). Environmental stress can accelerate *Arabidopsis* development and induce an ARR-like response in young plants similar to the ARR response seen in mature *Arabidopsis* plants. Little is known about the cell-signaling cascade or the molecular mechanisms involved in the ARR and ARR-like responses, thus mutants need to be identified in order to further our knowledge about these two responses. A classical mutant screen was performed on a population of 700 plants grown from neutron-mutagenized seeds. The mutant screen was performed 3.5 weeks post germination using plants that had been plate stressed. Plate stress involves allowing the seeds to grown on MS media for 15 days versus the usual 4-6 days. The plants were inoculated with 10^6 cfu/ml *Ps*, and symptoms were observed three days later. Potential mutants were chosen based on their diseased phenotype, in which chlorosis plus tissue damage was exhibited on all infected leaves. Eight potential mutants were identified and allowed to set seed. The identification of potential mutants will aid in gene identification, which will provide more insight into the ARR and ARR-like responses.

Razavi, Roxanne, Wei, Anhua and Chow-Fraser, Pat. McMaster University, Biology Department.

Assessing historical and present day fish habitat in the marshes of Point Pelee National Park

Point Pelee National Park, although most well known as a remarkable birding and butterfly attraction, is also recognized under the RAMSAR convention for its wetlands of international significance. These wetlands comprise over seventy percent of the Park’s area, and provide important habitat for many species of flora and fauna. Pelee has a rich history of human settlement and land-use dating back to the beginning of the 20th century. The levels of human use, however, were compromising the ecological integrity of the natural ecosystem, and subsequent management action was taken to buy back all the properties within Park boundaries. The effect of this change in land-use activity on marsh quality was investigated. An extensive database of historical fish surveys (1941-present) was used to determine the nature of the fish communities over time. The Wetland Fish Index (WFI; Seilheimer and Chow-Fraser, 2006), an assessment tool developed from 43 wetlands of Lake Ontario and Lake Erie, was used to infer historical conditions of Pelee marshes as fish habitat. Human impact was estimated by quantifying the total area occupied by buildings for a given year from available digitized aerial photos in a GIS. Changes in water levels were also used to assess the impact of natural fluctuations in marsh quality. Breaching periods are believed to introduce less nutrient-rich water into the wetlands, thereby improving the water quality of the eutrophic marshes. An overall recommendation for a water-quality monitoring plan for Pelee will also be presented.

Richardson, L. and Smith, Matthew D. Department of Biology, Wilfrid Laurier University.

Assembly of distinct Toc complexes in chloroplasts of *Arabidopsis thaliana*

Plastid differentiation and chloroplast biogenesis in particular, are important events in plant development. Chloroplast differentiation relies on successful import of many nuclear-encoded proteins. Initial steps of import involve preprotein recognition and translocation across the outer membrane, which are mediated by components of the Toc complex (translocon at the outer envelope membrane of chloroplasts), including two families of homologous receptors: the Toc159 and Toc34 GTPases. In *Arabidopsis*, members of the Toc159 receptor family (atToc159, atToc132, and atToc120) are thought to exist in free-soluble and membrane-associated forms, suggesting a possible model for protein targeting to the outer membrane. According to this model, Toc159 receptors recognize preproteins and target them to the outer chloroplast membrane via a GTP-dependent interaction with Toc34 family members (atToc33 and atToc34). Evidence also indicates that there is a preferential interaction between atToc159 and atToc33, and also between atToc132/120 and atToc34. To further demonstrate and characterize these preferential interactions, chloroplasts will be isolated from two *Arabidopsis* mutants, the atToc33 knockout mutant *ppi1*, and atToc34 knockout mutant *ppi3*, and used with *in vitro* translated, radiolabelled Toc159 family members for *in vitro* targeting assays. It is hypothesized that the targeting efficiency of atToc159 will be reduced in *ppi1* chloroplasts relative to wild-type chloroplasts, whereas the targeting efficiency of atToc132/120 will be reduced in *ppi3* chloroplasts compared to wild-type chloroplasts. This study will potentially provide further evidence for the preferential interaction between specific members of the Toc159 and Toc34 families in *Arabidopsis*, supporting the hypothesis that distinct Toc complexes exist for import of specialized groups of proteins.

Riddell, Heather and David Lesbarrères. Department of Biology, Laurentian University.

Differences in amphibian diversity and variation in individual size and mass between urbanized and protected wetland sites in the Sudbury area.

Habitat alteration and other anthropogenic influences on wetlands are known to have an adverse effect on amphibian populations often resulting in lower amphibian diversity. The aim of this study is to look at amphibian community and population characteristics with regards to habitat characteristics, following the hypothesis that human influence should have an impact at both levels. Amphibian population surveys were completed from May until August at two contrasting wetland sites in Sudbury, Ontario. First, the “Ponderosa” wetland on Junction Creek is a wetland impacted by municipal stormwater, industrial effluent, litter and other disturbances. Second, a control site has been surveyed in a protected conservation area. Frogs were captured and marked at night using nets and measures of snout-vent-length and mass were taken. Eight species were captured at the conservation area including green frogs (*Rana clamitans*), northern leopard frogs (*R. pipiens*), mink frogs (*R. septentrionalis*), spring peepers (*Pseudacris crucifer*), American toads (*Bufo americanus*), grey tree frogs (*Hyla versicolor*), wood frogs (*R. sylvatica*) and American bullfrogs (*R. catesbeiana*). All such species were captured at the disturbed site except the three latter species. The conservation area has greater species diversity but lower evenness than the “Ponderosa” wetland. *Rana sylvatica* and *H. versicolor* are often found in forested habitats and *R. catesbeiana* has a preference for deep water. The lack of both these habitat characteristics at the urbanized site is a potential explanation for the absence of these species. Furthermore, green frogs at the conservation area are smaller and lighter than those at the urbanized wetland. This result is explained by a difference in habitat composition at each site, including higher chemical concentrations at the urbanized site as well as habitat alteration from human settlement. Findings from this study show that wetland disturbance results in decreased amphibian diversity and that varying wetland conditions can impact amphibian growth.

Salehi, Parastoo. Department of Biology, McMaster University.

Effect of different diets on body mass of different specie of fruit flies

Drosophila, the most familiar fruit fly, is a small insect about three millimeters in length and two millimeters in width. *Drosophila* is one of the most valuable organisms in biological research particularly in genetics and developmental biology. *Drosophila* has been used as a model organism for research for a long time, and today several thousand scientists are working on many different aspects of the fruit fly. Many different factors can have an effect on *Drosophilas* bodies and their developments. One important factor is the diet. It has an effect not only on body mass, development time, and longevity of the flies but also influences some behavioral traits of the files, such as locomoter activities and aggressive behavior. The goal of my experiment is to discover the effect of different diets on body mass of different species of fruit flies. To achieve this goal, I used three different diets, and four different species. If body mass of each group is different than the other group in the same diet, then it means that the diet has different effect on different species of fruit flies. Another goal of my experiment is to discover the effect of different diets on longevity and viability of different species of fruit flies. In this case, I will measure the number of flies in a colony for each group. Number of the flies in each group could be an indication of viability and longevity of the flies.

Sanacee, May S., Department of Biology, McMaster University.

Adenovirus infection association with more rapid progression of Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a disease state characterized by cough and sputum production and irreversible destruction of pulmonary function that leads to significantly reduced airflow to the lungs. COPD is currently ranked as the fourth leading cause of death and is usually a result of cumulative exposure to tobacco smoke, occupational dusts or vapours, and indoor or outdoor air pollution. Fifteen per cent of all smokers develop COPD, indicating that other factors play a role in disease progression. The prevalence of an individual's previous adenoviral infection in this population was examined by designing a Polymerase Chain Reaction (PCR) assay targeted to the hexon gene (detects latent virus) and another targeting the E1A gene (detects virus incorporated into human DNA). These primers were designed using BLAST technology, BioSequencing software and OLIGO programs. The PCR for the hexon gene was perfected for sensitivity and specificity and used on 40 clinical samples. Probing techniques (Dot Blotting) was used to verify PCR results. There were 20 lung tissue samples and their corresponding blood tissue samples from patients who had consented to participating in this study. Of the 20 lung tissue samples, x tested positive for adenovirus and of the 20 buffy coat samples, x tested positive for adenovirus. Of the 20 patients, x tested positive for having adenovirus in both the lung tissue and buffy coat. In comparing these results to the degree of COPD severity, more evidence is provided suggesting that previous adenoviral infection can be involved in causing a more rapid progression of COPD.

Saravanan, A. , Burns, J.G. and H. Rodd. Department of Zoology, University of Toronto.

The effects of rearing environment on brain morphology and dendritic branching in the guppy (*Poecilia reticulata*)

Within-species differences in brain structure exist due to genetic differences, environmental effects or a combination of both. We are investigating the effects of environmental factors on brain structure by manipulating rearing conditions. The fish brain is a promising place to look for environmental effects because the processing of information occurs in more localized structures than in mammalian brains. Our prediction was that female guppies (*Poecilia reticulata*) raised in nature would have larger telencephalons, the region of the brain primarily responsible for spatial learning, relative to their laboratory-raised daughters. Brains were removed, photographed, and then morphologic measurements, such as maximum dimensions, area and volume were taken. Brain structures were bigger, by volume and maximum dimension in wild guppies relative to the lab-raised guppies suggesting that the guppy brain can be modified by rearing environment.

Studies on land vertebrates in the past have also shown that dendritic branching is associated with spatial learning. We were also interested in testing this idea on fish and hence extended our study to look at the effect of environmental factors on dendritic branching in the guppy brain. Our hypothesis is that enriched environments will provide a greater opportunity for spatial learning and may thus lead to a greater amount of dendritic branching. Based on this hypothesis, we predict that guppies raised in an enriched environment will have longer dendrites with a greater number of spines than their deprived counterparts. We are currently carrying out this experiment by raising female guppies in an enriched versus deprived environment in the lab. Their brains will be removed, sectioned and stained with Golgi Cox Solution, which can be used to detect neuron structure and measure the density of dendritic branching and dendritic spine counts.

Scattolon, Sarah A.^{1,2}, Jamal, Elizabeth M.¹, MacKenzie, Robyn N.², Perreault, Melissa², Szechtman, Henry², and Foster, Jane A.^{2,3} ¹Department of Biology, McMaster University, ²Department of Psychology, McMaster University, ³Department of Psychiatry & Behavioural Neurosciences, McMaster University, ⁴Brain-Body Institute, St. Joseph's Healthcare, Hamilton, ON

Regulation of MHC Class I genes in response to quinpirole-induced behavioural sensitization

Chronic administration of dopamine (DA) stimulant drugs, such as the D2/D3 receptor agonist quinpirole, results in *behavioural sensitization*, a phenomenon characterized by a progressive increase in the motor response to the drug. Behavioural sensitization is of interest because it may share underlying mechanisms with several psychopathological states in humans, ranging from drug addiction to obsessive-compulsive disorder. Because behavioural sensitization results in an enduring change in the nervous system, the mechanism of sensitization probably involves genes controlling neural plasticity. Here we investigate whether the newly identified class I Major Histocompatibility Complex (MHC) genes are involved in behavioural sensitization to quinpirole. We now know that class I MHC mRNAs and proteins are not limited to the immune system, but also expressed by CNS neurons following immune and non-immune challenges. In addition, neuronal expression of class I MHC is shown to play a functional role in neuronal plasticity in animal models. Our previous work has examined the effects of acute quinpirole on classical and non-classical MHC class I gene expression using quantitative real-time PCR. We observed a downregulation of class I MHC mRNAs in hippocampus, ventral tegmental area, and substantia nigra. The current focus is to detect changes in class I MHC mRNA expression in response to chronic quinpirole administration. We employed *in situ* hybridization to examine the cellular localization and expression levels of class I MHC mRNAs in prefrontal cortex, nucleus accumbens, ventral tegmental area and substantia nigra of sensitized animals (chronic quinpirole). Here, we observed persistent downregulation of non-classical class I MHC mRNAs in the ventral tegmental area and substantia nigra. The importance of these findings to behavioural sensitization is not clear but is interesting with respect to the non-immune functions of class I MHC in CNS neurons, and we speculate that class I MHC proteins may be associated with receptors, specifically those at synapses that are important to behavioural sensitization.

Schram, Amber. Department of Biology, McMaster University.

ATP/ADP Translocase in *Simkania negevensis* and *Waddlia Chondrophila*.

ATP/ADP translocase is a transport protein that is unique to *Chlamydiales*, *Rickettsiae* and plant plastids. ATP/ADP translocase allows these obligate intracellular bacteria to use the ATP produced by the host cell. It is a transmembrane protein that catalyzes the import of ATP from the host and the export of ADP from the bacteria to the host. This study was undertaken to determine if this gene is also present in *Simkania negevensis* and *Waddlia chondrophila*, which are both considered to be a member of the *Chlamydiales* based on their branching pattern in a 16SrRNA tree. A second objective was to determine the number of paralogs of this gene present in each of these species. This was done with the intention that the construction of a phylogenetic tree based on this gene would provide insight into the nature of the lateral gene transfer that is believed to have occurred between these groups. It was found by PCR amplification, using degenerate primers based on an alignment of the known *Chlamydiales* ATP/ADP translocase sequences, that *W. chondrophila* contains at least two paralogs of this gene in its genome. It was found that *S. negevensis* contains three copies of this gene, by BLAST searching the unfinished *S. negevensis* genome found on The Institute for Genomic Research (TIGR) website. The resulting phylogenetic tree, suggests that this gene was passed from a member of the *Chlamydiales* to the *Rickettsiae*. The phylogenetic tree also suggests that the ancestor of the *Chlamydiales* had at least two copies of this gene present in its genome.

Sebastian, Marissa, April Hayward and Jurek Kolasa. Department of Biology, McMaster University.

Data grouping method does not explain inconsistencies in the density-mass relationships found in aquatic and terrestrial ecosystems

The relationship between organism density and body mass has been a consistent source of discussion in the ecological literature for more than 30 years. There has been an on-going debate concerning the form of the mass-density relationship but two general patterns describing this scaling relationship have been found. In terrestrial ecosystems, population density often scales in proportion to body mass with a slope of -0.75, but the slope found in aquatic ecosystems is generally close to -1.0. We hypothesize that the inconsistency between the slopes found for terrestrial and aquatic systems is due to the fact that terrestrial ecologists tend to group data

according to taxonomy, whereas aquatic ecologists tend to group data according to the size of individuals. We tested whether data grouping method could account for the variation in the slope observed in terrestrial and aquatic systems using invertebrate density and body size data collected from natural aquatic microcosms in Jamaica. When data were grouped according to taxa the slope of the density mass relation was -0.22 ($r^2 = 0.03$, $p > 0.05$). When the same data were grouped according to size class, the slope of the relation was -0.14 ($r^2 = 0.031$, $p > 0.05$). Our results are inconclusive, but generally do not support the hypothesis that differences in slopes observed in aquatic and terrestrial systems result from differences in data grouping method. The absence of significant relationships between density and body size found here may result from the narrow range of body sizes considered in this study (<3 orders of magnitude).

Seetharaman, Ashwin. Department of Biology, McMaster University, Hamilton.

A comparative analysis of the genes involved in the germ line proliferation and differentiation in nematodes

Nematodes are widely used as model organisms in various fundamental and applied biological research. My study focusses on understanding evolutionary divergence in the genes involved in the germ line proliferation and differentiation between 3 closely related nematode species namely *C. elegans*, *C. briggsae* and *C. remanei*. A comprehensive knowledge on the level of divergence between the three nematode species would help us get a better understanding of the changes in the mechanism of germ line proliferation and differentiation. My plan of research involves cataloging the genes involved in the germ line proliferation and differentiation in *C. elegans*, identifying orthologs of those genes in *C. briggsae* and *C. remanei*, determining the percentage of identity and similarity between the orthologs and predicting the possible changes in terms of the gene functioning and testing such predictions by RNAi experiments. The percentage of identity and similarity between the gene orthologs in the three nematode species was estimated using the BLAST program which compares any two given protein and DNA sequences to calculate identity/similarity between them. This analysis coupled with the RNAi knockdown experiment will help us understand the evolutionary changes in the gene signalling pathways in these three nematode species.

Shrivastava, Sneha. York University.

Cross-talk between leptin and insulin signaling pathways - Role of SOCS-3 and its subsequent effect on fatty acid metabolism

Obesity had become a worldwide health problem as it is a major risk factor in the development of insulin resistance, the hallmark of type 2 diabetes, and its prevalence has increased dramatically in recent years. Fat tissue has been identified as an endocrine organ that produces many hormones and factors, including leptin. Leptin is now known to regulate peripheral metabolism along with regulating appetite. Also, it has been shown that human obesity is associated with increased plasma leptin levels. Recently, the suppressor of cytokine signaling 3 protein (SOCS-3) has been implicated in crosstalk between the leptin and insulin signaling pathways, leading to the impairment of leptin action. The purpose of this study was to examine the effect of chronic insulin treatment on leptin signaling pathway in L6 rat skeletal muscles which might be mediated through SOCS-3. Here it is demonstrated that insulin significantly increases SOCS-3 expression in L6 rat skeletal muscle cells. Furthermore, chronic insulin treatment reduced the leptin induced phosphorylation of ACC, ObR and IRS-1. Both acute leptin (60nM) and chronic insulin (100nM) treatment individually increased fatty acid uptake in L6 cells and chronic insulin treatment significantly decreased fatty acid oxidation. However, L6 cells those which were pre-treated with insulin demonstrated no change in fatty acid uptake and fatty acid oxidation when treated with leptin. In summary, it is demonstrated here that leptin's action is impaired in the presence of insulin through the induction of SOCS-3.

Sitarz, Eva. Department of Biology, Wilfrid Laurier University.

Characterization of a cell line derived from the brain of goldfish *Carassius auratus*

Ex-vivo culture of nervous tissue cells from various organisms has proven useful in several areas of research including aquatic toxicology and neurobiology. Cell cultures are used to study the functions of neurons and glial cells and also to test potential neurotoxicant compounds. Fish brains, unlike mammalian brains have been shown to display extensive adult neurogenesis and post-injury repair, thus facilitating development of fish brain cell lines. Understanding the mechanisms behind these novel capabilities could be very useful in biomedical research. Cell cultures were derived from brain tissues of adult goldfish (*Carassius auratus*) and maintained in a growth medium consisting of Leibovitz's L-15 supplemented with 10% fetal bovine serum (FBS). Conditions

for optimal growth and maintenance of differentiated characteristics will be investigated. Physiological effects will be noted when neurons are exposed to high ammonia levels. Glial cells will be identified by immunostaining for glial fibrillary acidic protein (GFAP), a marker for glial cells. Neuronal cells will be identified by presence of neurofilament (NF). Presence or absence of N-methyl-D-aspartate (NMDA) receptors, characteristic of neural cells, will also be investigated by immunocytochemistry. The convenience and ready availability of a well characterized fish brain cell line could greatly advance studies in comparative neurobiology and neurotoxicology.

Smith, Melanie P., Maxwell, Denis P. and Kohalmi, Susanne E., Dept. of Biology, Univ. of Western Ontario
Expression Analysis of Six Arogenate Dehydratase Genes in Arabidopsis

In plants, phenylalanine is required for protein synthesis as well as the synthesis of key secondary metabolites, including lignin and various flavonoids. Phenylalanine biosynthesis in plants is commonly believed to use the arogenate pathway, where arogenate dehydratases (ADTs) catalyze the final reaction converting arogenate into phenylalanine. A family of six *ADT* genes has been identified in the *Arabidopsis* genome and we hypothesize that members of this gene family may show differential tissue and developmental expression patterns in contributing to the plant's overall its phenylalanine requirement. To analyze gene expression we utilized RNA blot hybridization to quantify the level of transcript (mRNA) for each *ADT* gene in different plant tissues.

In silico analysis revealed that the six ADTs identified have a conserved domain structure: an N-terminal putative signal peptide, an internal catalytic domain, and a C-terminal ligand binding or ACT domain. To ensure that hybridization probes selectively bind to a single *ADT* sequence, computer analysis identified the signal peptide region as having the lowest similarity. Primers were designed to specifically amplify the signal peptide region and to generate DIG-labeled probes by PCR. To test if each probe only binds to its corresponding *ADT* sequence, plasmids containing each a single *ADT* sequence were used as a template for a Southern hybridization. A blot containing samples for all 6 *ADTs* was probed and re-probed six times demonstrating that each probe only recognized a single ADT. For the Northern hybridization, total mRNA was extracted from roots, leaves, stems, flowers and, siliques. An initial hybridization indicates that the *ADT1* expression might be extremely low in all *Arabidopsis* tissues analyzed. RNA blot hybridization using all six *ADT* probes will provide evidence if these genes are differently expressed giving us a new and comprehensive insight in how these genes might be regulated on the transcriptional level.

Song, Catharine and Saleh, Mazen T. Department of Biology, Laurentian University.

***in silico* Comparison of Microbial Exportomes**

A significant number of completed eubacterial and archaeal genome sequences are presently available. These organisms cover a wide range of habitats and include free-living and parasitic, pathogenic and non-pathogenic, extremophiles and autotrophs. Therefore a unique opportunity exists in which to compare their proteomes. This study was undertaken to specifically compare and contrast bacterial exportomes, that fraction of the proteome with predicted N-terminal signal sequences. The exportome includes all extracytoplasmic proteins, including the secretome. A total of 176 prokaryotic proteomes were examined using the *ExProt* program. Over the entire range of organisms tested, archaeal exportomes were the smallest, followed by those from Gram-positive organisms. Gram-negative organisms have the largest exportomes. In the Gram-negative organisms only, there is a positive correlation between proteome size and exportome size. Exportome size does not distinguish between pathogenic and non-pathogenic prokaryotes. For its proteome size, *B. burgdoferi* has an unusually large number of lipoproteins, contributing to a relatively large exportome. However, its exportome is smaller than that of *T. pallidum*, a closely related spirochete with a similar proteome size. Of the Gram-positive organisms tested, *Mycoplasma* proteomes deviate from the pattern observed by showing closer similarity of their signal sequences to those of Gram-negative organisms. In the archae, *M. kandleri* and the pyrococci share certain features of their signal peptides not common to other archae.

Spakowski, A., and Skandalis, A. Department of Biological Sciences, Brock University.

The Effect of Mutated XPG on Splicing of POLB and HPRT in Humans

Previous studies have proposed that, upon encountering a DNA lesion, RNA polymerase II either pauses or incorporates a mutated base into the mRNA molecule leading to the generation of an aberrant transcript. To investigate the validity of this proposal, splice variants of the POLB and HPRT loci were isolated and characterized from cells possessing a mutated XPG protein. XPG is necessary for transcription-coupled repair, which is responsible for the removal of DNA lesions. Thus, cells lacking functional XPG possess a greater

number of DNA lesions than the control cells. The type and number of splice variants identified in human skin fibroblasts lacking XPG (AG08803) were compared with splice variants previously characterized from human fetal lung fibroblasts (MRC5). An increase in the type and frequency of splice variants present in AG08803 was considered an indication that errors by RNA polymerase II contributed to the production of aberrant transcripts.

Tien, Jean C., Stone, John R, and Poinar, Hendrik. Department of Biology, McMaster University
Telomere degradation as a mechanism of species extinction.

In 2004, Reinhard Stindl published a paper that proposed that telomere erosion is a mechanism of species extinction. This hypothesis is based on the assumption that telomeres shorten due to extremely low levels of telomerase during the first cell cycle of fertilized ova in plants and animals. According to Stindl's paper, telomeres are expected to lose 10bp every other S-phase of the cell cycle, approximately 5bp per generation. This mechanism would limit the duration of species and its effect was coined by Stindl as the "species clock". If the species clock were a real phenomenon, then telomeres would shorten to a critical length after thousands of years, depending on generation times and initial telomere lengths of species. To test Stindl's hypothesis, DNA extracted from blood samples was analysed to determine telomere lengths for extant Elephantidae (Asian and African elephants). We hope to compare these telomere lengths to the average telomere length determined from DNA extracted from mammoth tissues from Siberia. Then determine an expected time of extinction for mammoths using the species clock, and compare the expected time of extinction with the estimated time of extinction, determined on the basis of carbon dating. The expected and estimated times of extinction should be within range of each other for Stindl's hypothesis to remain unfalsified. Assuming that extant elephants were and are more successful (or less endangered) than were mammoths, we expect to see shorter telomeres in the mammoth sample. To provide another means for testing Stindl's hypothesis, telomere lengths of a variety of mammals were analysed using a simple computational model to generate typical extinction times, which were compared to species lifetimes derived from fossil evidence.

Thillainadesan, Gobi and Kohalmi, Susanne E. Department of Biology, University of Western Ontario.

Expression of select ADTs as GFP fusion constructs to determine subcellular localization in *Arabidopsis*.

Phenylalanine (Phe) is an essential aromatic amino acid required for protein biosynthesis in all organisms. In plants, Phe also acts as a precursor for many secondary metabolites such as lignin, flavonoids, melanins, phenols and tannins. However, only bacteria, fungi and plants are capable of synthesizing Phe *de novo*. In plants, Phe is believed to be synthesized using the arogenate pathway that requires the enzymes prephenate aminotransferase (PAT) and arogenate dehydratase (ADT) to convert phenyl-pyruvate to arogenate and phenylalanine, respectively. Six genes have been identified in *Arabidopsis thaliana* as putative ADT coding sequences. *In silico* analysis reveals that all six ADTs share a similar overall protein structure consisting of a putative N-terminal signal peptide, an internal catalytic ADT domain and a C-terminal ACT domain, a putative ligand binding domain for post-translational feedback regulation. According to computer based predications the signal peptides direct the ADT proteins to the chloroplast. This is consistent with the hypothesis that Phe biosynthesis can occur in chloroplasts. Such N-terminal signal peptide sequences are typically cleaved upon entry of the organelle to release an active mature protein. To determine the subcellular localization of ADT1 and ADT3, GFP-ADT fusion constructs have been designed either allowing expression of the full-length ADT sequences or as ADTs lacking the N-terminal putative signal peptide. These constructs are designed for transient expression in *Arabidopsis* so that GFP expression can be assayed and the subcellular localization of the ADT enzyme can be visually determined *in vivo*.

Thakur, Ajit¹ and ²Fradin, Cecile. ¹Department of Biology, ²Department of Physics and Astronomy, McMaster University.

Nuclear Transport Cargos in live *C. elegans* oocytes

Nuclear transport of targeted cargo is a fundamental biological process common to all eukaryotes which ensures the proper trafficking of macromolecules between the cell cytoplasm and nucleus. We are investigating the nuclear import of molecular cargos bearing the classical SV40 large T-antigen NLS (Nuclear Localization Signal). Because we are interested in the motion of the cargos before they reach the nuclear envelope, we chose as a model the oocyte of the nematode species *C. elegans*. The large size of these cells allows us to easily interrogate them with imaging and optical techniques. Transgenic worms expressing GFP-NLS (Green Fluorescent Protein-NLS) and GFP-NLSm (mutated NLS) at the oocyte stage are created by microinjecting

DNA containing the sequence for these fusion proteins directly into the parent worm's gonad arms. The merits of various techniques that allow us to characterize and quantify the motion of fluorescent particles will be discussed. For this study we chose to use a non-invasive technique called Fluorescence Correlation Spectroscopy, which is based on the analysis of the temporal fluctuations of the fluorescence signal. This method will allow to test the diffusive nature of the nuclear transport process before the cargo reaches the nuclear envelope.

Tomczyk, Katarzyn. McMaster University.

Neurosphere Proliferation Patterns

Neural stem cells when isolated from the brain and will grown in the presence of mitogens will expand as neurospheres. These spheres are a heterogeneous mixture of neuronal and glial progenitors as well as stem cells. To determine whether the expansion of these spheres follows a specific pattern, cells were isolated from murine neural tissue and cultured to the 3rd passage. The cells were plated at (20 cells/ μ l of the culture media) to produce the neurospheres and treated with 5-Bromodeoxyuridine (BrdU) at different stages of neurosphere development. Actively dividing cells will incorporate the BrdU into their DNA as a replacement for thymine residues during the S-phase of division. Neurospheres were treated for 24 hours with BrdU at 4, 6 and 8 days after plating. Following the BrdU treatment the neurospheres were fixed, sectioned and treated with a fluorescent secondary antibody to visualize the BrdU+ labeled cells. A general nuclear stain (DAPI) was applied to sections of the spheres to demonstrate all cells in the sphere. We found that the BrdU in the culture media induced differentiation in the neurospheres in comparison to BrdU negative controls. Considerable heterogeneity was observed in the number of dividing cells within the neurospheres and no pattern of BrdU expression was detected.

Tracey, Brady J.A., Department of Biology, McMaster University.

Analysis of Gene Paralog Divergence in African Clawed Frog genus *Xenopus*

Allopolyploidism in African Clawed frogs (genus *Xenopus*) allows for the studying of gene divergence after genome duplication. The goal of this work is to better understand the timing and frequency that genes become nonfunctional, remain redundant; become subfunctionalized, or acquire novel function following duplication. We applied statistical method to compare molecular changes in a polyploid species to those in a diploid species. In expressed duplicates, we theorized that novel function generally did not occur in a "burst" immediately after gene duplication - the "neofunctionalization early" hypothesis. Based on knowledge of the time frame of speciation and allopolyploidization events in the genus *Xenopus*, we compared molecular changes at early and late points in time following duplication. Certain retained duplicates have similar rates of non-synonymous substitution, which suggests that expression is often not triggered by mechanisms that necessitate novel function.

Vafaie, F. and Guglielmo, C.G. Department of Biology, University of Western Ontario.

The effect of diet nutrient composition on aerobic enzyme activities in flight muscle of a migratory bird, the yellow-rumped warbler (*Dendroica coronata*)

Dietary nutrients (fat, protein, carbohydrate) are known to affect exercise performance and the mixture of metabolic fuels used during exercise in humans. Previous research with birds suggests that among species the fuels used during migratory flight are affected by diet, with insectivores using relatively more protein and frugivores using more fat. However, some bird species, such as yellow-rumped warblers (*Dendroica coronata*) are known to change their diet seasonally between insects and fruit and it is not known how this might affect their fuel use during migration. In a previous experiment Yellow-rumped warblers were maintained in captivity and fed synthetic diets for one month that were formulated to mimic insects (32% carbohydrate, 40% protein, 9% fat) or fruit (73% carbohydrate, 16% protein, 5% fat). In exercise trials the fruit birds were able to maintain higher average VO₂ than insect birds. Fruit birds also had higher plasma concentrations of non-esterified fatty acids and triglycerides during exercise indicating that they used more fat than insect birds. My objective was to determine whether the observed differences in exercise performance and fuel use were related to differences in the activities of metabolic enzymes in the muscles of these birds. Citrate synthase was measured as an indicator of aerobic capacity, and β -hydroxyacyl-CoA-dehydrogenase and carnitine palmitoyl transferase were measured as indicators of fatty acid oxidation potential. The effects of body mass, body fatness and diet on these enzymes, and their relationship to individual exercise performance will be presented.

Vander Heyden, Chris. Department of Biology, Lakehead University.

An ecophysiological investigation of snow bank fungi from western North America.

Snow bank fungi are an interesting but little studied group of macrofungi (e.g. gilled mushrooms, bracket fungi, puffballs and cup fungi) which form their fruiting bodies beside melting snow (or soon thereafter) in the mountains of western North America. Tissue cultures were derived from eight different species (*Calbovista subsculpta*, *Calvatia fumosa*, *Clitocybe albirhiza*, *Clitocybe glacialis*, *Neohygrophorus angelesianus*, *Nolanea holoconiota*, *Plectania nannfeldtii*, *Sarcosoma mexicana*). An investigation was done on their growth responses to various temperatures, selected enzyme capabilities, and potential ecological status of each as either a wood and litter decomposer or as being ectomycorrhizal. All species exhibited growth at 5°C, and have an optimum between 15°C and 20°C but grew poorly or did not grow at all at 30°C. This growth rate pattern to temperature is typical of other psychrotolerant fungi. Cultures were tested for the production of the following enzymes: cellulose, amylase, pectinase, laccase and tyrosinase. Different patterns were observed among the eight species but most isolates produced pectinase, amylase and tyrosinase. The wood block decomposition experiment and the ectomycorrhizal synthesis experiment with jack pine seedlings are still in progress but results will be available in time for this presentation. Regardless of ecological status, these fungi have adapted to withstand cooler temperatures in order to take advantage of available moisture each spring from melting snow in order to fruit. Such ability allows for successful fruiting and spore dispersal every year in comparison with late summer and autumn fruiting macrofungi which may fruit only once every 5 or 10 years when moisture is plentiful.

Van Es, Sarah J. and Lee, Lucy E.J. Department of Biology, Wilfrid Laurier University.

Effects of the amoeba *Neoparamoeba pemaquidensis* on rainbow trout gill cells.

Neoparamoeba pemaquidensis (NP) is a marine protozoan believed to cause amoebic gill disease (AGD) in several fish species. The objective of this study was to determine how NP affects the morphology, epithelium integrity, and protein expression of rainbow trout gill cells, using an *in vitro* model. Changes in gill cell morphology were qualitatively observed using phase contrast microscopy. Epithelium integrity was assessed by measuring changes in resistance of a gill cell monolayer to an electrical current as exposure time to NP increased. Two-dimensional gels were run so that expressed proteins could be compared between control cells and amoeba-exposed cells. Cells exposed to NP changed shape, fragmented, and detached from the flask bottom. A decrease in trans-epithelial resistance to current of NP-exposed gill cells in transwell cultures was seen, suggesting that the amoebas may exert their pathogenic effects by damaging the gill epithelium. Differences in protein expression were seen between control and treated gill cells. These protein differences may reflect physiological changes caused by NP. Certain proteins that were detected only in infected cells may have been expressed as a defense response, while NP may have inhibited the expression of proteins detected only in control cells. The changes seen in morphology, epithelium integrity, and protein expression suggest that NP is virulent to cultured rainbow trout gill cells. This study therefore affirms that rainbow trout gill cells are useful models for studying AGD.

Van Kempen, Tonia N. , Demmer, Crystal M. * and Litzgus, Jacqueline D. Department of Biology, Laurentian University.

Comparison of turtle species abundance and richness in damaged and recovered lakes in Sudbury, Ontario

Due to a long history of mining activity, the Sudbury area has been subjected to intense environmental damage. Local terrestrial and aquatic ecosystems have been damaged by atmospheric deposition of acid and metal contaminants. Since turtles live across both these ecosystems, they can serve as valuable bioindicators of overall environmental health. We tested the hypothesis that anthropogenic pollution would have a negative impact on turtle populations marked by low abundance and species richness in damaged lakes. Over a five month period, three damaged eutrophic lakes and three recovered oligotrophic lakes were sampled using Fyke nets, dip nets and visual surveys. We found the opposite of what we predicted. Turtle species richness was low in both lake types and included only three species; the painted turtle (*Chrysemys picta*), the common snapping turtle (*Chelydra serpentina*) and the Blanding's turtle (*Emydoidea blandingii*). The Blanding's turtle was found in a single damaged lake. Overall, the abundance of all species was higher in the damaged lakes than in the recovered lakes, which is likely attributable to the greater productivity of these eutrophic lakes. The painted turtle populations in two of the damaged lakes were large enough for demographic analyses; interestingly, both lakes exhibited a female-skewed sex ratio. In urban settings, turtle populations typically display male-biased sex ratios as a result of female mortality from vehicular traffic during nesting migrations. Our unusual findings

require further investigation. This ongoing study will provide baseline information for future population and turtle species assemblage research.

Vincent, Ashlee, M. Department of Biology, McMaster University.

Mating-type specific *Sxi1α* and *Sxi2a* genes influence ploidy in *Cryptococcus neoformans* var. *neoformans*
Cryptococcus neoformans is a basidiomycetous, heterothallic fungal pathogen. This species has two sexes distinguished by their mating type (MAT) locus, MAT α and MATa. Among other genes, MAT α is characterized by having the *sxi1α* gene while MATa by having the *sxi2a* gene. Previous research has found both *sxi1α* and *sxi2a* genes to influence sexual development. Here I show that both genes influence the stability of ploidies in *C. neoformans*. Six artificially synthesized diploid strains of *C. neoformans* (2 wildtype, 2 Δ *sxi1α*, 1 Δ *sxi2a*, and 1 Δ *sxi1α*/ Δ *sxi2a* strain) were tested under two temperature (RT and 37°) and medium (YEPD and SD) conditions. Preliminary results show that in all 4 conditions, the largest percentage of haploids was recovered in double deletion strains, followed by the *sxi2a* deletion strain, the *sxi1α* deletion strains, and the wild type strains. Among the four growth conditions, YEPD/RT showed the highest frequency of haploid cells while SD/37° showed the lowest. The demonstration that both genetic and environmental factors can influence diploidy stability should allow further tests of various hypotheses about the evolution of haploid and diploidy.

Wang, Xin. Department of Biology, York University.

The effect of ectopic expression of superoxide dismutases (SODs) on aging in *Drosophila melanogaster*
 Reactive oxygen species (ROS) have been suggested to be an important effector in aging and various diseases. Superoxide dismutase (SOD) as the frontline scavenger for superoxide (O₂⁻) has been drawing great attentions. Numerous genetics studies have assisted our understanding of SOD functions on the lifespan determination in *Drosophila*, at the same time, suggesting the possible tissue-specific effect on aging. In this study, the impact on lifespan from ectopic alteration of SODs activity was investigated. The GAL4/UAS binary expression system, RNA interference (RNAi) based gene silencing and sense-transgene based overexpression were employed to achieve silencing and overexpressing SOD1 and SOD2 respectively. Five GAL4 drivers were tested and their expression patterns were partially characterized by GFP reporting system and immunohistochemistry. The results confirm the important role of SOD on aging in *Drosophila* and suggest that two important factors in oxidative stress mediated aging are the tissue-specific SOD activity and the presence of a small number of cells potentially crucial in the aging.

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The use of inducible phenotypic plasticity to elucidate paradoxical mechanisms involved in snail radular growth

Freshwater apple snails, *Pomacea bridgesi*, were used to study the inducible characteristics of temperature and diet and their effect on radula growth rate and pattern. The snails were allowed to thrive in 4 different environments for 4 weeks, then subjected to a cold shocking procedure to determine growth rate. The first attempt at this failed, with all snails dying during the cold shocking procedure. Their snail radula were used to analyze radular shape and pattern differences due to changing environments. A second experiment with a lower temperature cold-shocking procedure was used to allow an analysis of snail growth rate in different treatments. Findings show a distinct difference in radula growth rate, shape and pattern due to diet and temperature. The analysis is used to determine if these are independent effects or if combined they produce different results thereby indicating and interaction of factors. The results obtained were then used to infer potential heritability of radular growth traits and gain insight into the mechanism by which radular growth occurs while snails constantly feed. This information was used to further decipher the mechanism of growth. Prior research shows gastropod radula are produced by odontoblasts on the posterior end of the radula sheath into transverse rows in an almost conveyor-belt like manner. The growth mechanism is similar to that of a belt sander in the way that the teeth are constantly moving to grind food but still able to replace themselves. This paper attempts to verify this mechanism and add to its understanding. When combined with recent studies of genetic markers in snails, the analyses presented aid in associating phenotypic plasticity and genes. The results found in this study help to solve the paradox of snail radular growth and bring us a step toward creating a coherent understanding of radular growth.

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The Effect of Single Nucleotide Polymorphisms on the Efficiency and Efficacy of V(D)J Recombination

Vertebrate immune system is maintained by two-tier systems, innate immunity and adaptive immunity. Adaptive immunity depends on the enormous diversity of B-cell receptors and T-cell receptor that interacts with antigens. V(D)J recombination initiates this process of diversification by dynamically recombining Variable (V), Diversity (D) and Joining (J) gene segments on antigen receptor locus. A number of proteins and protein complexes are implicated in this process, including RAG (Recombination Activating Gene)-1/2 protein and non-homologous end joining (NHEJ) proteins. The single nucleotide polymorphisms (SNP) of the proteins involved in V(D)J recombination were examined for their potential effect on the efficiency and efficacy of V(D)J recombination. Various non-synonymous SNPs were found in protein-encoding regions of many proteins and were also predicted to be deleterious to the protein functions by bioinformatics tools. Two of the single nucleotide polymorphisms on RAG-1 protein were synthesized by site-directed mutagenesis of wild-type RAG-1 encoding plasmid. We are currently attempting to express RAG-1 proteins containing a SNP (or wild-type) in 293T and M12 cell lines along with wild-type RAG-2. The comparison of the efficiency and the efficacy will be made by comparing the rate of recombination and joining of the substrate plasmid.

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